EFFECTS OF ALCOHOL ON THE HEART

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Declaration

I declare here that the work for this thesis was carried out by my self or under my direct supervision.

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Dedication

I dedicate this work to my most respected mother Mrs. Ayesha Haque, my father Mr. M A Haque, my beloved wife Rehana and my two dearest lovely sons Asmar Hussaini and Asif Hussaini.

A N Hussain Ahmed

Abbreviations

ATPase	Adenosine Triphosphatase
BV	Blood Vessel
Ca	Calcium
Cm	Centimeter
CT	Connective Tissue
CV	Coefficient of Variation
D	Diameter
EEL	External Elastic Lamina
EDTA	Ethylenediamine Tetra Acetic Acid
EVG	Elastic Van Geison
FFA	Free Fatty Acids
Gm	Gram
H & E	Haematoxylin and Eosin
Ht.	Heart
IA	Intimal area
IBAS	Interactives Bild Analysen System
IEL	Internal Elastic Lamina
IT	Intimal Thickness
K	Potassium
KAT	Kontron AT
Kg	Kilogram
LA	Luminal area
LV	Left Ventricle
MA	Medial area
Max	Maximum
Mg	Magnesium
Min	Minimum
MT	Medial Thickness
Na	Sodium

No.	Number
NS	Not Significant
P	Probability
PAS	Periodic Acid-Schiff
RGB	Red Green Blue
RV	Right Ventricle
sd	Standard Deviation
Sig.	Significance
VDU	Visual Display Unit
μm	Micrometer
Wt.	Weight

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Abstract

A series of randomly collected human hearts, removed at autopsy from decedents known to be chronic alcoholics were examined macroscopically, histologically, and morphometrically. In the latter context an accurate, reproducible, computer-assisted semi-automated method was utilised to carry out several measurements of cardiac myocytes, interstitial connective tissue and blood vessels. Control hearts were collected, preserved, and examined histologically and morphometrically in an identical manner.

Hearts in alcoholics were enlarged and heavier than controls when adjusted for body weight and other confounding factors. Fixed heart weights were significantly lower than the fresh heart weights in both groups. The proportion of the fat component of the heart was significantly higher in alcoholics than controls. Although the gender ratio differed significantly in the two groups because of the criteria used for selection and exclusion, it was concluded that this was not likely to have caused group differences through confounding. A significant degree of excessive interstitial fibrosis was also noted within the myocardium of the alcoholics compared to controls. No significant vascular abnormalities were noted particularly in the micro-circulation. It was also shown that in chronic alcoholics there are cardiac myocytic changes which include an increased diameter of myocytes and of their nuclei. This myocytic hypertrophy together with increased interstitial fibrosis contributed to the cardiac enlargement in alcoholics and may eventually lead to cardiac dysfunction.

The incidence of chronic alcoholism is on the increase world-wide and sudden death related to cardiac problems in association with the chronic abuse of alcohol is a well known occurrence, whose pathogenesis has been poorly established in clinical and animal studies. Within the limitations of the material available for this study, this work enables a clearer insight into the potential direct relationships between alcohol and the cardiac myocyte, and into the pathogenetic mechanisms of alcohol-related cardiac problems.

CHAPTER 1

Introduction

Over the years an association has been postulated between the excessive consumption of alcohol, particularly over a number of years, and malfunction of the cardiovascular system, particularly of the heart itself. This correlation has been explored from both the clinical and the pathological aspects, and in addition, experimental work has been conducted on animals to attempt to assess more directly the pharmacological effects of alcohol on the heart and the circulation.

The aim of this chapter is to review the historical background and the current concepts of alcohol induced heart disease, in particular the role of alcohol in the context of physiological, clinical and pathogenetic mechanisms associated with heart disease.

1.1 Historical Background

Chronic excessive consumption of alcohol as a causative factor in heart failure was first mentioned by Wood in 1855. Walshe (1873) described a form of "*localised cirrhosis in the ventricles and columnæ carneæ*" in the heart of chronic alcoholics. Bollinger (1884) in his description of the "*Münchener Bierherz*", drew attention to the effects of beer on the heart. Sansom (1892) considered alcohol as a cause of heart disease and Steel (1893) was of the opinion that alcohol was a common cause for "*muscle failure of the heart*". Tresilian (1898) reported additional cases of heart failure associated with chronic alcoholism. Osler (1912) stated "*the heart and arteries in chronic topers show degenerative changes*". While Price (1922) listed alcohol as a cause of fatty infiltration, fatty degeneration, chronic interstitial myocarditis, hypertrophy and dilation of the heart. However, alcohol was not recognised as a cardiotoxin for many years because most instances of alcoholic heart disease were felt to be secondary to malnutrition (Aalsmeer and Wenkebach, 1929; Weiss and Wilkins, 1936 and 1937; Blankenhorn, 1945; Blankenhorn et al, 1946). Brigden (1957) in the United Kingdom and Burch and Walsh (1960) in the United States were primarily responsible for calling attention to alcohol as a cause of cardiomyopathy. Thus, it was

eventually realised that many patients with alcohol-related heart disease did not display the typical features of nutritional heart disease and did not respond to nutritional replacement.

Mackenzie (1902) was the first to use the term "*alcoholic heart disease*" and Graham Steel (1906) found myocardial failure in beer drinkers in association with peripheral neuritis. Mackenzie (1908) postulated that arsenic in beer was the cause of heart failure. Subsequently, these reported examples of congestive heart failure in chronic alcoholics were attributed to nutritional deficiencies rather than to alcohol itself.

Aalsmeer and Wenkebach (1929) described the cardiac manifestations of Oriental beriberi, and thereafter physicians recognised this condition in the West as being mainly the result of alcoholism. It was thought that heart failure seen in alcoholics occurred as the result of thiamine deficiency. Subsequent reports further suggested that heart disease in chronic alcoholics was associated with thiamine deficiency (Keefer, 1930; Benchimol and Schlesinger, 1953) or with cobalt added to beer (Little and Sunico, 1958; Bonenfant et al, 1967; Hall and Smith, 1968; Kesteloot et al, 1968; Alexander, 1972).

Many clinical studies have established the concept of cardiotoxicity of alcohol and this has led to a marked interest in experimental and clinical research into acute and chronic effects of alcohol on the cardiovascular system (Haggard et al, 1941; Eliaser and Giansiracusa, 1952 & 1956; Frederiksen and Hed, 1958; Evans, 1959; 1961 and 1964; Hicke and Hall, 1960; Goodwin et al, 1961; Fowler et al, 1961; Burch et al, 1964; Carnevali, 1964; Hibbs et al, 1965; Dines, 1965; Burch and DePasquale, 1968 and 1969).

1.2 Pathogenetic Mechanisms

The direct toxic effect of alcohol on the heart has been established both in experimental animal (Burch et al, 1971, Vasdev et al, 1975) and in man (Regan et al, 1966; Regan, 1971; Friedman and Leiber, 1977). The effect of alcohol will vary depending upon previous exposure and the presence of pre-existing cardiac disease. The effect of alcohol on the heart appears to relate to the blood levels of alcohol, and

the myocardial depression produced is reversible within a few hours folowing consumption (Regan, 1971). In chronic alcoholics larger doses are required to produce an effect (Regan et al, 1966; Wong, 1973). However, if an attack of heart failure or other cardiac complication has occured, further exposure to alcohol is likely to produce a similar response more readily (Regan, 1971; Timmis et al, 1975).

In order to understand the pathogenesis of alcoholic heart disease the metabolic and pathological effects of alcohol on the heart and the myocardium in particular are reviewed.

1.2.1 Acute Myocardial Metabolic Effects

Acute alcohol exposure contributes to depression of the myocardial metabolic function and this may have a metabolic basis, in particular, there may be a response to an effect on Ca^{++} movement (Seeman et al, 1971) or from changes in calcium flux at the microsomal level (Retig et al, 1977). There are in addition, alterations in the active transport of potassium and sodium across the cardiac myocyte's cell membrane (Israel et al, 1970). Calcium binding and uptake by cardiac microsomes are also impaired by lethal doses of ethanol (Swartz et al, 1974). This also appears to be a basic mechanism of the effect of alcohol on any cell. In high doses alcohol may produce a transient loss of potassium and phosphate out of the cardiac muscle cell (Regan et al, 1963).

Ethanol decreases the duration of the action potential in the myocardium. This effect is concentration-dependent and seems to reflect the physical alterations in the cell membranes occurring when ethanol interacts with the hydrophobic region of the lipid bi-layer of the sarcolemma (Williams et al, 1980).

Alterations in lipid transport in the myocardium are now well recognised (Regan et al, 1966). Alcohol reduced the uptake of free fatty acids (FFA) by the left ventricle. The increased triglyceride uptake that results promotes an accumulation of the lipid in the myocardium. Using histopathological techniques Ferrans et al (1965) demonstrated substantial lipid deposits in the alcoholic heart. The reduced extraction of free fatty acids may relate to reduced arterial level substrate as well as from competitive inhibition from increase extraction of acetate.

Further metabolic consequences of alcohol ingestion include increased production of lactate and acetate and their increased utilisation by the heart (Lindeneg et al, 1964; Kako, 1973). It has also been found that the chronic intake of large amounts of alcohol results in a leakage of isocitrate and malic dehydrodenase into the coronary sinus regardless of the presence of clinical heart disease; simultaneous decrease of FFA extraction occurs without alteration in the triglyceride extraction (Lindeneg et al, 1964). Acute exposure to ethanol decreases the activity of some plasmamembrane-bound enzymes (Williams et al, 1975), inhibiting the oxidative enzymes of mitochondria (Bing, 1978). The utilisation of fatty acids is impaired but their esterification to triglycerides increases (Kako et al, 1973).

It has been suggested that the toxic metabolites of alcohol, particularly acetaldehyde, are also important in the pathogenesis of the myocardial damage (Kikuchi and Kako, 1970), and that, in addition, a direct release of noradrenaline from the myocardium may further accentuate the myocardial changes (James and Bear, 1977). Acetaldehyde has also been shown to affect the mitochondrial membranes and mitochondrial function (Rubin et al, 1970) and myocardial protein synthesis (Schreiber et al, 1972; 1974).

Acute administration of ethanol causes morphological changes in mitochondria and sarcoplasmic reticulum seen under electron microscope (Klein and Harmjanz, 1975), but does not detectably alter myocardial structure as seen by light microscopy. These changes in the myocardial cells are reflected in increases of the serum enzymes (Lott and Stang, 1980). These ultrastructural changes can partially be prevented by vitamin E pre-treatment, suggesting that lipid peroxidation contributes to the myocardial toxicity of ethanol (Redetzki et al, 1983).

The myocardium is relatively rich in catalase (Herzog and Fahimi, 1976), although mammalian heart muscle lacks alcohol dehydrogenase and the microsomal alcohol-oxidising system, which has been shown to protect the myocardium from the deleterious effects of alcohol in prolonged experiments on the rat heart (Kino, 1981), but there is no available data suggesting an acute protective effect.

1.2.2 Chronic Myocardial Metabolic Effects

Although it is possible to demonstrate acute effects of alcohol on the myocardium the relationship of these to the pathogenesis of the clinical cardiac disease is less clear. It has indeed been suggested that at least 10 years of chronic alcohol exposure is required before the development of alcoholic heart muscle disease (Levi et al, 1977).

Alcohol has been found to have an effect on the myocytic contractile proteins and their regulatory enzymes. It may also bring about an alteration in glycoprotein metabolism and an alcian positive staining material may be detected in the muscle (Regan et al, 1974).

Extracellular accumulation of sodium and water has been demonstrated using chromium 51 EDTA but no changes in the potassium content were shown (Thomas et al, 1980). It is possible that this effect results from an alteration in the membrane phospholipid concentration which thus limits the entrance of sodium and water into the cell. Increased sarcolemmal-myofibrillar distance has been observed in electron microscopic studies (Alexander, 1967). These changes may limit the availability of calcium to the contractile proteins. Membrane function and permeability may be impaired by hydroxyl radicals (Slater, 1984). Studies by Hess et al (1981) have shown that the super-oxide anion radical depresses Ca^{++} uptake and Ca^{++} ATPase activity. Free radicals alter myocardial excitation-coupling and contractile protein function (Tepper et al, 1986).

The experimental changes that have been observed closely resemble the myocardial damage which is seen in pre-clinical alcoholic heart disease in the human (Gould et al, 1969; Spodick et al, 1972; Wu et al, 1976).

Light and electron microscopic studies in animals given chronic alcoholic treatment have shown myocardial cell abnormalities, including dilation and swelling of sarcoplasmic reticulum (Regan et al, 1975; Thomas et al, 1980) and intercalated discs (Sarma et al, 1976), the presence of glycoproteinaceous material between cardiac muscle fibres (Regan et al, 1975), alterations in mitochondria (Sarma et al, 1976), and triglyceride deposits within the myocardial cell (Regan et al, 1974). A modest hypertrophy may develop in the alcoholic rat heart (Whitman et al, 1980), but the larger animals do not show such a change (Regan et al, 1974; 1975). The biochemical

changes with chronic use include increased left ventricular collagen (Thomas et al, 1980), diminished ß–oxidation of fatty acids (Edes et al 1983), depressed mitochondrial respiration (Regan et al, 1975; Sarma et al, 1976; Whitman et al, 1980), decreased intracytoplasmic calcium transport (Sarma et al, 1976, Noren et al, 1983), depressed myofibrillar ATPase activity (Noren et al, 1983), impaired protein synthesis (Schreiber et al, 1982), and a relative preponderance of glycolysis over glycogenolysis (Edes et al 1983). Chronic alcohol ingestion also alters the constituents of biomembranes: it increases both phospholipid methylation (Prasad and Edwards, 1983) and synthesis of phospholipids rich in saturated fatty acids (Littleton et al, 1980).

Recently, the production and accumulation of fatty acid ethyl esters have been demonstrated in the ethanol-exposed heart (Lange and Sobel, 1983). These compounds derive from esterification of free fatty acids and may induce mitochondrial dysfunction by being hydrolyzed to a toxic free fatty acid. Another new finding is that myocardium of several mammalian species contains catalase and that prolonged ethanol consumption may increase its activity (Fahimi et al, 1979). Furthermore, when ethanol was given to rats with inhibited catalase activity, extensive structural abnormalities were observed in the myocardium (Kino, 1981). This suggests that myocardial catalase may protect the heart from the cardiotoxicity of ethanol.

The density of cardiac ß-adrenergic receptors is decreased in chronic alcoholic rats but increased during the early withdrawal period (Banerjee et al, 1978). The decrease of cardiac ß–adrenergic receptors suggests augmented catecholaminergic stimulation during chronic alcohol use. Interestingly, the hypertropic response seen in alcoholic rat heart correlates significantly with increases in urinary excretion of adrenaline and noradrenaline (Adams et al, 1986).

1.3 Physiological Effects of Alcohol

1.3.1 Experimental Effects in Animals

Ethanol exerts an acute negative inotropic effect on isolated strips of atrial and ventricular myocardium (Mason et al, 1978). The depression of contractility is dependent on ethanol concentration and appears when the concentration exceeds

about 100 mg/dl. The inotrophic influence of different alcohol parallels the length of their carbon side chain i.e. their lipophilic activity (Nakano and Moore, 1972). Animal experiments have also shown myocardial depression after ethanol administration (Regan et al, 1966; Horwitz and Atkins, 1974; Goodkind et al, 1975; Stratton et al, 1981). In some studies, ethanol was shown to impair cardiac performance even at concentrations as low as 110 to 120 mg/dl (Regan et al, 1966; Horwitz and Atkins, 1974).

Exposure of rats to 25 per cent ethyl alcohol can be shown to produce depression of the peak isometric systolic tension (Maines and Aldinger, 1967) and in some studies increased myocardial irritability and disturbances of cardiac rhythm were also noted. More recently it has been shown in dogs in whom alcohol provided 36 percent of total daily calorie intake, when given short term (18 months) and long term (52 months), would cause an increase in the left ventricular end-diastolic pressure and this was associated with an increase of the end-diastolic and decrease in stroke volume (Thomas et al, 1980). Increased left ventricular collagen was the apparent basis for the compliance abnormality. However, when the velocity of the contractile element (used as an index of LV contractility) was measured, the changes were only significant in the group which had had long term exposure (52 months) suggesting that the altered contractility related to the availability of calcium to the contractile proteins (Thomas et al, 1980). In another study, dogs were fed ethanol for 29 months: in vitro studies of glycerinated cardiac muscle fibres demonstrated reduced contractility. However, in vivo functional abnormalities were not present (Sarma et al, 1976). Progressive impairment of myocardial function in ethanol infused dogs were related with myocardial damage and the mitochondria appear to be the most vulnerable organelle seen under electron microscopy (Jones et al, 1975).

Hearts from rats fed ethanol for periods varying from 12 weeks to 12 months have been studied either as isolated preparations (Whitman et al, 1980; Segel et al, 1981; Chan and Sutter, 1982) or in situ (Hepp et al, 1984). No abnormalities in basal cardiac function have been shown. However, perfused hearts have shown decreased left ventricular response to challanges with dobutamine (Segel et al, 1981), increased afterload (Whitman et al, 1980), electrical pacing (Chan and Sutter, 1982). After ethanol was withdrawn for 4 to 6 months cardiac response to dobutamine was no more significantly different from controls (Segel et al, 1981). The exact biochemical mechanisms responsible for the impaired contractile function of the chronic alcoholic heart have not been identified.

It is important to recognize that none of the chronic ethanol experiments using adult animals has been able to produce a model of congestive dilated alcoholic cardiomyopathy. In a recent study, new born domestic turkeys were fed ethanol for 8 weeks resulting in enlargement and hypokinesis of the left ventricle compatible with incipient dilated cardiomyopathy (Noren et al, 1983). This study, however, failed to provide data to indicate a congestive state.

1.3.2 Effects in Man

Acute administration of alcohol in normal man produces an increase in cardiac output and heart rate without altering the stroke volume (Riff et al, 1969), but others reported no significant changes in cardiac output and stroke volume (Wendt et al, 1966). The acute administration of alcohol resulted in a significant decrease in cardiac output and stroke volume and increase in myocardial oxygen consumption (Ganz, 1963). The volume effect of alcohol on the submaximal exercise test is insignificant but myocardial oxygen consumption increases, possibly indicating a reduction of mechanical efficiency (Mitchell and Cohen, 1970). Similarly, in patients with fatty livers, alcohol administration induces a depression of left ventricular (LV) function with an increased in the left ventricular end-diastolic pressure and a decrease in stroke work in response to aortic pressure (Regan et al, 1969). Diminished ventricular performance in response to exercise has also been observed in cirrhotic patients without evidence of cardiac disease (Gould et al, 1969). Non-invasive systolic time interval studies in asymptomatic alcoholic subjects have also shown some impairment of left ventricular function (Spodick et al, 1972; Wu et al, 1976). A schematic representation of the some of the pathogenetic mechanisms of alcohol and acetaldehyde on the myocardium is shown in figure 1.3.1 derived from Richardson and Wodak, 1983.

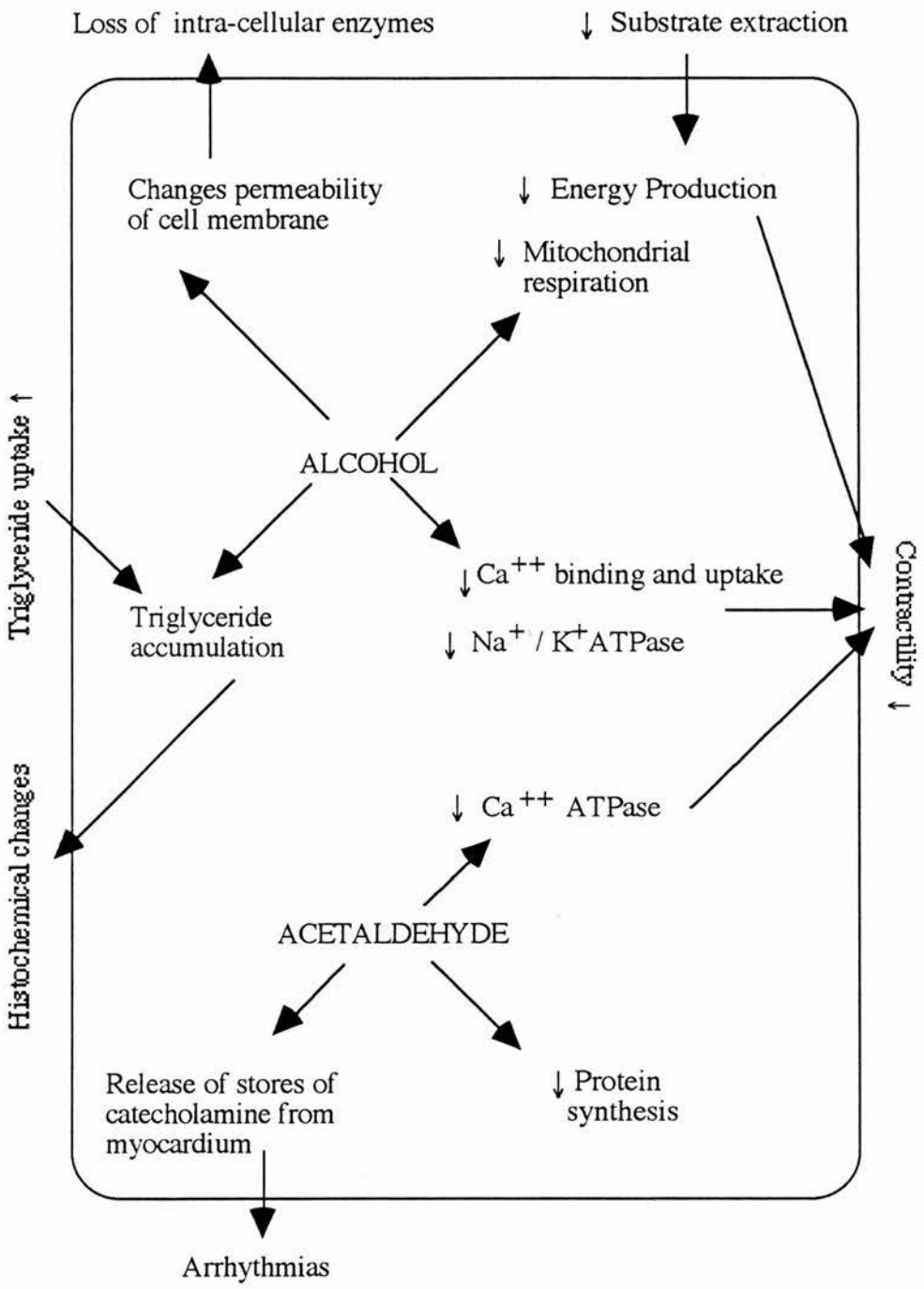

Figure 1.3.1: Summary of some of the pathogenetic mechanisms of alcohol and acetaldehyde on the myocardium (derived from Richardson and Wodak, 1983).

The results of the human studies are conflicting. Studies using cardiac and peripheral vascular catheterization in healthy subjects have shown that modest ethanol intake either does not affect or improves left ventricular pump function (Gould et al, 1971). Non-invasive imaging in similar subjects has revealed either impairment (Delgado et al, 1975; Lang et al, 1985) or no changes (Child et al, 1979; Kupari, 1983) in left ventricular ejection performance. In patients with stable coronary, valvular or pericardial heart disease, early invasive data (Gould et al, 1971; 1972) suggested an acute myocardial depression from rather tiny amounts of alcohol. Measurements of the systolic time intervals (Ahmed et al, 1973) have consistently demonstrated post-ethanol increases in the pre-ejection period and its ratio to left ventricular ejection time both in normal and in cardiac patients. This pattern of change has been interpreted as indicating ethanol-induced depression of myocardial contractility.

Recent research strongly suggests that these changes reflect decreased preload rather than impaired myocardial performance (Kupari, 1983). It is important to recognise that ethanol is also an arterial and venous dilator (Altura and Altura, 1982) and a diuretic (Nicholson and Taylor, 1938). By these effects, it can change cardiac loading conditions and pump function even without affecting myocardial performance. The importance of the peripheral effects is nicely demonstrated in a study which showed that, in patients with severe heart failure, alcohol can evoke an acute relief of pulmonary congestion in association with a tendency to improvement in cardiac pump function (Greenberg et al, 1982).

Many of the human studies have thus failed to show any myocardial depression after alcohol intake, some even during the stress of isotonic (Riff et al, 1969) or isometric exercise (Kupari et al, 1983). At first this may seem irreconcilable with the in vitro data about negative inotropic effect of ethanol. It seems very likely that in an intact organism the vasodilating and sympathomymetic effects of ethanol outweigh its myocardial depressant capacity at low blood concentrations. The reductions in peripheral arterial resistance and left ventricular size unload the heart, while the increased sympathetic activity and secretion of adrenaline simultaneously counteract the ethanol-induced impairment of myocardial contractility (Kupari, 1983). When the contribution of these effects is excluded, by autonomic blockade and controlling the loading conditions, the negative inotrophic influence of ethanol can be demonstrated also in man (Lang et al, 1985).

1.4 Clinical Cardiovascular Disorders

Chronic ethanol abuse has been associated with a variety of cardiovascular disorders (Koide et al, 1972; and Koide and Ozeki, 1974), ranging from sudden death (Wannamethee and Shaper, 1992) to chronic non-ischaemic heart disease accompanied by long-standing cardiac failure (Hicks et al, 1993); furthermore, it is the major cause of dilated cardiomyopathy in the western world (Clark 1984; Walsh and Vacek 1986). Indeed, cardiovascular disease is now generally accepted as one of the major causes of morbidity and mortality in chronic alcoholics (Hennekens et al, 1978; Dyer et al, 1981; Fraser and Upsdell, 1981; Ashley and Rankin, 1980; Deutscher et al, 1984; Criqui, 1987; Rosengren, 1987 and 1988; Ohara et al, 1989; Shaper, 1990; Pinn and Bovet, 1991; Marmot and Brunner, 1991; Beaglehole, 1992; Day et al, 1993; Douds and Maxwell, 1994).

Congestive cardiomyopathy related to alcoholism is also considered as the most frequent identifiable cause of cardiomyopathy with its incidence ranging from 21% to 32% in the two series reported from referral centres (Fuster et al, 1981; Schwartz et al, 1984;). Its incidence is even higher in situations where there is a high frequency of ethanol addiction (Regan, 1990).

In addition, a high incidence of asymptomatic cardiac abnormalities, which may precede clinical decompensation, has been described (Schenk and Cohen, 1970; Askanas et al, 1980; Mathews et al, 1981; Kino et al, 1981; Steinberg and Hayden, 1981; Kelbaek et al, 1984; Ahmed et al, 1984).

In the asymptomatic subject, a variety of investigative techniques have established that alcohol abuse is associated with sub-clinical depression of left ventricular function. Current data suggest that alcoholic patients without clinical evidence of heart failure may have, on investigation, reduced diastolic compliance, manifested as an elevated end-diastolic pressure and diminished end-diastolic volume (Regan et al, 1969; Ahmed et al, 1980). Contractility and relaxation indices are also impaired (Ahmed et al, 1980). Similar features were observed in the experimental situation and, in a canine model using chronic ethanol administration, reduced diastolic compliance occurred without cardiac hypertrophy and before basal contractility was affected (Thomas et al, 1980). These changes suggest a similarity to left ventricular responses to chronically elevated systolic blood pressure (Mathews et al, 1981).

Recently, a relatively homogeneous group of alcoholic subjects who were employed and living with their families was assessed. Detailed evaluation of their nutritional state revealed no evidence of malnutrition in spite of their chronic misuse of alcohol. Radionuclide ventriculograms showed dysfunction of the heart in as many as one third of these chronic alcoholics, with concomitant abnormalities of skeletal muscle in almost half. The deleterious effects of alcohol were apparently dose-related, and injury to the heart and skeletal muscle often co-existed in the same patients (Marquez et al, 1989).

Cardiac decompensation was shown to occur typically in men between 30 and 55 years of age who had ingested at least 80 grams of alcohol almost every day for a minimum of ten years (Burch and Giles, 1971). The physical signs of cardiac decompensation found in these individuals were similar to those observed in other forms of congestive cardiomyopathy with low cardiac output heart failure. In a number of these patients, cardiomegaly could be observed either due directly to the effects of alcohol or due to mitral regurgitation developing as a consequence of papillary muscle weakness. A common complication of cardiomyopathy is the development of pulmonary or peripheral arterial emboli. Systemic emboli can originate from mural thrombi forming within the left ventricle and left atrium. Pulmonary emboli are often associated with mural thrombi in the dilated right side of the heart or with thrombophlebitis in the venous system (Regan, 1990).

1.5 Arrhythmias

A variety of atrial arrhythmias in alcoholic subjects with and without overt cardiomyopathy or an enlarged heart have been described during acute intoxication (Koskinen et al, 1990; Kupari and Koskinen, 1991; Koskinen and Kupari, 1992). In a report of patients admitted to an emergency department, 32 separate symptomatic dysrhythmic episodes that required hospitalisation occurred in 24 patients who drank habitually and who had also indulged in recent heavy ingestion before the arrhythmia (Ettinger et al, 1978). Atrial fibrillation was the most common arrhythmia; plasma electrolyte levels were usually normal. Sinus rhythm was restored spontaneously in some patients but the restoration usually required cardioversion or pharmacological intervention. After the normal sinus rhythm was restored, moderate conduction delays could be found by electrocardiography during high-speed recording. These

ECG changes were considered to be the background for the induction of acute arrhythmias.

This phenomenon has been reproduced in the electrophysiology laboratory. In 11 subjects with clinical evidence of heart disease who abused alcohol, atrial fibrillation was inducible without acute ethanol administration in 4 subjects, and in an additional 4 subjects only during its use (Engel and Luck, 1983).

In an analysis of new-onset atrial fibrillation in 40 patients under 65 years of age, alcohol was considered to be causative or contributory in approximately two thirds of the patients (Lowenstein et al, 1983).

Relative risk of acute arrhythmias in the persons who reported six or more drinks per day as compared to persons who reported drinking at least monthly, but less than daily was at least doubled for atrial fibrillation, atrial flutter, supraventricular tachycardia and atrial premature complexes (Cohen et al, 1988).

Ectopic ventricular beats can be observed in the early phases of alcoholic detoxification but, although sinus tachycardia is observed occasionally, ventricular tachycardia is rarely recorded. One explanation is that ventricular tachycardia in alcoholic patients rapidly progress to fibrillation (Ziln et al, 1980).

Several reports from forensic medical sources have documented a higher incidence of sudden death without known heart disease than previously appreciated in subjects who abuse ethanol (Kramer, 1968; Randall, 1980).

A prospective study of sudden death at the Pathology Institute in Moscow revealed that 17% of all cases were related to alcohol abuse, predominantly in patients under 50 years of age (Vikhert et al, 1986). The cases studied were associated with instantaneous death (within 30 minutes of symptoms). None of these could be attributed to sleep apnoea in that death occurred in the street, in the office, or in other public places in the presence of witnesses. Significant coronary disease was absent in these patients, but evidence of cardiomyopathy by light and electron microscopy was present in specimens taken within hours of death. Histochemical analysis of autopsy myocardium indicated a reduction of mitochondrial enzymes, particularly succinic dehydrogenase, thought to be relatively specific for alcoholic cardiomyopathy of the idiopathic variety. These studies give no information about the relative purity of the

alcohol consumed by these patients; this is important, since contaminants may contribute to cardiac pathophysiology.

Similar findings were shown in Sweden in a study of persons registered as alcoholic (Wilhelmsen et al, 1973; Rosengren et al, 1987, Rosengren and Wilhelmsen, 1987; Lithell et al, 1987; Rosengren et al, 1988), in a five year study in Finland (Suhonen et al, 1987) and in a study in female alcohol abusers in Rochester, Minnesota, USA (Beard et al, 1986).

The chronic alcoholic canine model was also studied in this respect, ensuring that the animals were well nourished throughout the experimental period. Low-grade prolongation of conduction time in the left ventricle was shown. Abnormal diastolic compliance occurred within 12 months and was associated with the accumulation of fibro-connective tissue in the myocardial interstitium (Patel et al, 1989).

1.6 Alcohol and Coronary Artery Disease

A series of epidemiological investigations have suggested that low to moderate ethanol intake reduces the risk of coronary heart disease (Yano et al, 1977; Turner et al, 1981; Cullen et al, 1982; Colditz et al, 1984; Gordon and Doyle, 1985; Klatsky et al, 1986; Friedman and Kimball, 1986; Stampfer et al, 1988; Klatsky et al, 1990; Klatsky, 1990; Boffetta and Garfinkel, 1990; Rimm et al, 1991; Jackson et al, 1991; Suh et al, 1992; Garg et al, 1993; Gaziano et al, 1993; Shaper et al, 1994). Furthermore, there is evidence to suggest that consumption of ethanol in low to modest quantities may actually decrease the incidence of death due to myocardial infarction (Leger et al, 1979; Kozararevic et al, 1980; Kono et al, 1991). In a longitudinal study on physicians followed up since the time of their undergraduate years, moderate alcohol drinking was shown to have little adverse effect on health (Thomas et al, 1980). Ingestion of modest quantities of ethanol may increase high-density lipoproteins and protect against development of coronary atherosclerosis (Barboriak et al, 1979; Suh et al, 1992). On the other hand, evidence suggests that severe alcoholism may favour development of coronary heart disease (Dyer et al, 1981; Rosengren et al, 1987). Recently the presence of severe coronary artery disease in a non-smoker who drank 1 to 4 martinis per day was reported (Nethala et al, 1993).

Other evidence suggests that patients who ingest even small quantities of alcohol may have an increased incidence of cerebrovascular disease (Yano et al, 1977), although this may also be associated with an increased tendency toward hypertension in such persons (Kozararevic et al, 1980). Many chronic alcoholics are also heavy cigarette smokers (Klatsky et al, 1981; Rosengren et al, 1988). Cigarette use may not be essentitial to the genesis of cardiomyopathy in alcoholics (Ahmed et al, 1985). A patient with severe alcoholism who has acute myocardial infarction runs a greater chance of dying than a patient who does not drink heavily (Deutscher et al, 1984). Alcohol intemperence is strongly associated with an increased risk of sudden death after myocardial infarction (Lithell et al, 1987). It seems clear that mortality in alcoholics who have cardiovascular disease is more than in non-alcoholics (Suhonen et al, 1987). On the other hand, the claim that ethanol ingestion may reduce the incidence of myocardial infarction remains to be proved. Recent report controlling for age, suggests that heavy drinkers are more likely to be admitted to a general hospital for myocardial infarction and other cardiovascular disorders compared with abstainers (Chick et al, 1986).

Of interest is the 17 year follow-up experience of nearly 2000 white men originally aged 40 to 55 years from the Western Electric Co. Chicago, USA (Dyer et al, 1980). When mortality rates were adjusted for age only, total intake of ethanol at the level of six or more drinks per day was associated with an increased risk of death from all types of cardiovascular disease, coronary heart disease, or cancer. The association between alcohol intake and death from cardiovascular and coronary heart disease failed to persist after adjustments were made for other risk factors such as smoking and raised blood pressure. The association between excessive alcohol intake and raised mortality was generally stronger for those deaths that occurred more than 10 years after entry in the study. A British prospective study carried out over a six year period also found no clear relationship between the amount of alcohol intake and major ischaemic heart disease (Shaper et al, 1987).

An earlier Swedish report from Gothenburg, had indicated that alcohol abuse was an independent risk factor for death from coronary artery disease and that this increase was limited to patients with evidence of coronary disease but without recent myocardial infarction (Wilhelmsen et al, 1973).

1.7 Hypertension and Alcohol

Acute ethanol administration or ingestion appears to lower blood pressure (Altura and Altura, 1987) but there is an abundance of epidemiological evidence showing that chronic ethanol consumption increases blood pressure (Yamada et al, 1991). In alcohol misusers the proportion of patients who are hypertensive may be as high as 50% (Saunders et al, 1981). Blood pressure was significantly higher even among light drinkers (<20 ml/day) compared to non-drinkers (Wakabayashi et al, 1994). "Social" drinking of alcohol is often associated with a small rise in systolic blood pressure but, in those subjects who habitually imbibe heavily, the rise of blood pressure may be substantial (Cregler et al, 1989). A study was undertaken on alcoholics with healthy hearts to assess the effects of inebriation and post-intoxication period on the level of arterial pressure in relation to cardiac function, as compared with recovery levels. The hypertension was not related to a high output state, and peripheral arterial resistance was substantially elevated (Regan et al, 1986). High plasma levels of aldosterone and renin, as well as urinary catecholamines, correlated with this vasoconstrictor response and the decline of the levels of hormones as blood pressure spontaneously normalised is compatible with this interpretation. Whether this normal system is altered between episodes of active heavy drinking is not known. Moderate, habitual drinkers have normal activity of these hormones (Arkwright et al, 1982). The response of the smooth muscle to adrenergic stimulation may vary with different stages of alcohol intake and an increased sensitivity to the neurotransmitter noradrenaline may have contributed to the observed rise of peripheral vascular resistance (Altura et al, 1980).

The incidence of chronic essential hypertension in those who abstain for a year is similar to that in age-matched controls (approximately 10%) [Dunn et al, 1977]. Essential hypertension in active alcoholics appears less responsive to antihypertensive agents (Saunders et al, 1982). Recently the INTERSALT study reported a significant relationship between heavy drinking (3-4 or more drinks/day) and blood pressure which has been observed in both men and women (Marmot et al, 1994).

1.8 Diagnosis of Alcohol-Related Heart Disease

In examination of biopsy specimens from patients or autopsy tissue preparations, distinctive features have been found to be lacking in alcoholism with alcoholic heart disease, as compared with findings in those with other causes of congestive cardiomyopathy (Goodwin, 1974; Olsen, 1979; Richardson and Wodak, 1983; Robbins et al, 1994).

Ultrastructurally, early in the pre-failure stages there would appear to be dilatation of the sarcoplasmic reticulum and the undifferentiated portion of the intercalated disc (Ettinger et al, 1976), but these events apparently are obscured at later stages when considerable myocytolysis may be seen. Increased amounts of fibrous tissue may appear as an increase in the interstitial collagen component or replacement of myocardial fibres. The diagnosis of alcoholic heart muscle disease can now be confirmed by taking particular note of the detailed drinking history, myocardial enzyme analysis, with histological, histochemical and ultrastructural changes indistinguishable from dilated cardiomyopathy (Richardson and Wodak, 1983) the measurement of tissue enzyme activities in suspected alcoholic heart disease may enable confirmation of the diagnosis. Decreased concentrations of mitochondrial enzymes, particularly succinic dehydrogenase, have been reported to distinguish alcoholic cardiomyopathy from the idiopathic dilated form (Vikhert et al, 1986).

1.9 Causation of Alcoholic Heart Disease

It is not certain whether ethanol itself or the presence of other substances in alcohol-containing beverages causes the damage to the heart or whether these changes are the consequences of deficiency of substances in the body caused by the nutritional deprivation associated with chronic abuse of alcohol (Gould, 1970; Regan, 1971). The role of malnutrition and vitamin deficiency have been considered but preclinical cardiac abnormalities in alcoholics have been shown to be independent of nutritional status and vitamin deficiency (Dancy et al, 1985; Regan and Morvai, 1987). Alcohol may also act indirectly through its primary metabolite acetaldehyde (James and Bear, 1977; Kupari and Suokas, 1989). Magnesium deficiency (Fankushen et al, 1964; Lim and Jacob, 1972) and altered catecholamine metabolism (Adams and Hirst, 1986;

Malpas et al, 1991) have also been implicated. Deficiency of other essential metals are no more likely to be involved (Bogden et al, 1984).

An immunological basis has also been considered, but only a minority of patients with alcoholic cardiomyopathy have circulating muscle-specific antimyolemmal antibodies that are commonly found in post-myocarditis cardiomyopathy (Maisch et al, 1983). A genetic predisposition has been considered but no significant differences found compared to controls (Kachru et al, 1980).

Many studies have shown that alcohol produces a number of biochemical, electrophysiological and morphological changes in the cardiac muscle cell and its sub-organelles. These changes may produce an acute negative inotropic effect and they probably underlie the pathogenesis of alcoholic cardiomypathy in chronic alcohol abusers. The report of elevated blood acetaldehyde and acetate levels in chronic alcoholics aroused interest about the role of the metabolites of alcohol-derived acetaldehyde and acetate, and it seems that, under certain circumstances, both can contribute to the acute effects of drinking (Nuutinen et al, 1983 and 1985).

1.10 Myocardial Small Vessel Disease in Chronic Alcoholism

Additionally, the proposal has been advanced that the pathological changes in chronic alcoholic heart disease are not specific to alcohol toxicity, but may instead reflect the effects of chronic ischaemia (Burch et al, 1971; Morin and Côté, 1972).

Most pathological studies of alcoholic heart disease have focused on the light and electron microscopic alterations of the muscle fibre. If the myocardial alterations in alcoholic cardiomyopathy are secondary to the effects of chronic ischaemia, however, it is conceivable that these changes are mediated by disease of the small coronary vessels because large coronary vessel disease is usually absent (Brigden, 1972). However, little attention has been paid to the small vessels of the myocardium.

Several clinical and experimental studies have pointed to the existence of small vessel disease in chronic alcoholism. In 1953, in a study of chronic alcoholics, Benchimol and Schlesinger described oedema of coronary vessels in an early stage of the disease. Shiian, in 1961, found a marked increase in vascular permeability

together with changes in vascular tone in chronic alcoholics. Increased vascular permeability is responsible for the interstitial oedema in the myocardium and in the vessel walls. Protein- rich plasma accumulates between the layers of the arterial wall, producing gradual narrowing and distortion of the vessel lumen. This can progress to complete occlusion with subsequent focal myocardial necrosis; the latter heals leaving a small scar, so commonly observed in cases of alcoholic cardiomyopathy. If a sufficient number of sections are examined, one or more microscopic foci of fresh myocardial necrosis may be seen; these are sometimes followed by dystrophic calcification.

It is of interest that somewhat similar changes have been produced experimentally in dogs rendered hypomagnesaemic over a long period of time (Wener et al, 1964), and that hypomagnesaemia has been reported in chronic alcoholics (Martin and Bauer, 1962). Pintar and his associates (1965) described significant changes in small intramyocardial vessels of three chronic alcoholics dying of cardiomyopathy. The authors observed vascular oedema and degeneration, disorganisation of the vessel wall layer and deposition of PAS positive material in the subintima. Sohal and Burch (1969), in an experimental study, fed mice a diet containing 15 percent ethanol by volume for three months. They noted ultrastructural changes in myocardial capillaries consisting of swollen and degenerating endothelial cells and narrowing of the vascular lumen. The authors speculated on the role of chronic hypoxia induced by the vascular alterations in the pathogenesis of alcoholic heart disease.

In the study of Burch and Giles (1977), the small coronary arteries of two patients with alcoholic cardiomyopathy were studied by means of fine particle barium injection and soft x-ray technique. The spatial architecture of these small arteries, which penetrated the depths of the right and left ventricular myocardium, were normal. Their lumina were somewhat dilated and their luminal surfaces were smooth. The "tree circulatory units" were described from this study and were found to be normal. The left ventricular myocardium was richly supplied with small arteries, whereas the myocardium of the right ventricle was not so richly supplied. The myocardium of the papillary muscles and the trabeculæ carnæ of the left ventricle also had a good supply of small arteries, as did the remote endocardial myocardial appendages. Numerous fine hair-like arteries extended to the epicardial myocardium of the right ventricle to form a brush-like appearance.

Although there is, as yet, no conclusive evidence for the direct toxicity of alcohol on the heart, the existence, severity and extent of small vessel disease in the hearts of chronic alcoholics has been evaluated by Factor (1976) to determine the significance and degree of microvascular alterations if any of the pathological changes in alcoholic cardiomyopathy could be attributed to the vascular abnormalities.

1.11 Vascular Abnormalities

Vascular changes were tabulated in chronic alcoholics by Factor (1976) in the following categories, each of which included alterations of varying degrees of severity.

A. Vascular oedema - This group included vessels demonstrating intracellular vacuolisation, extracellular vacuoles and microcysts, and loosening and disorganisation of the vascular wall layers. The presence of proteinaceous fluid, or blood constituents such as leukocytes, erythrocytes, or platelets within the vascular wall were also tabulated under this heading.

B. Vascular sclerosis - This category comprised vessels with intimal and medial hyperplasia and/or fibrosis, elastic tissue proliferation and the deposition of PAS positive material in the vessel wall.

C. Perivascular fibrosis - Concentric perivascular hyaline fibrosis and an increase in loose perivascular connective tissue were placed in this category.

D. Vascular inflammation - This included the presence within the vascular wall of obvious acute or chronic inflammatory cells, or cells assumed to be inflammatory in type e.g. Anitschkow myocytes.

E. Subendothelial humps - This referred to the presence beneath the endothelium of an asymmetric accumulation of smudgy eosinophilic material, usually acellular and variably compact, which protruded into the vascular lumen.

Other variables were noted but not formally tabulated. These included the presence of interstitial fibrosis not directly related to the vessels, areas of myocardial

fibrosis, interstitial inflammation, epicarditis, recent or acute thrombosis of vessel and areas of acute myocardial infarction.

The most frequent alteration noted was vascular oedema, which was present in 48% of the vessels from all nine alcoholic cases as compared with an incidence of 26% in the control group. The changes consisted of both intracellular and extracellular oedema and empty-looking vacuoles were frequently noted within the cells of the intima and media.

More significant was the presence of oedema, which appeared to be extracellular resulting in separation of individual cells within the vessel wall by clear spaces; this was often seen to extend into the media. In the vessels in which these changes were more pronounced and confluent, marked separation and disorganisation of the vessel wall layers was seen. Occasionally, subendothelial microcysts were seen and these were often filled with eosinophilic material. Infrequently, the latter contained erythrocytes or leukocytes. Irregular cystic spaces were also noted in the media of several larger vessels (50μm to 200μm) and these contained basophilic material, most likely representing intercellular ground substance.

Perivascular fibrosis was the second commoner abnormality and occurred in 42% of the tabulated vessels in the alcoholic group and 13% in the control group. Interstitial and myocardial fibrosis were associated frequently with it. The perivascular fibrosis was predominantly of two types. Loose, poorly organised fibrous connective tissue extended from the adventitia to involve the myocardial fibres adjacent to the interstitial compartment. Such fibrosis was often associated with increased numbers of chronic inflammatory cells, extravasated erythrocytes, and haemosiderin-laden macrophages within the perivascular interstitium, but no active vasculitis was noted. Denser, more organised perivascular fibrosis when seen consisted of concentric, hyalinised, acellular fibrous tissue, but was usually not associated with increased interstitial inflammation. It was commonly seen within papillary muscles, but was also scattered throughout other part of the myocardial sections examined. Although not tabulated separately from the looser, less organised fibrosis, the concentric fibrosis generally was the less common of the two forms.

Vascular sclerosis was noted in 36% of the vessels examined in the alcoholic group, compared to 15% of the vessels in the control group. Several types of abnormality were apparent. Subendothelial intimal thickening was noted, similar in

composition and staining characteristics to the subendothelial humps described below. The major difference, however, was that the sclerosis was not nodular but was a linear intimal thickening occurring along one portion of a vessel wall. Asymmetric medial sclerosis was also noted, in which intimal and medial fibrosis occurred along a short segment of the vessel wall. This type of sclerosis usually did not markedly encroach upon the vascular lumen. Major encroachment and narrowing of the lumen was often present when the sclerosis affected the entire vessel wall in a symmetrical fashion. Trichrome stains revealed an increased connective tissue within the sclerotic vessel walls. Stains for elastic tissues revealed reduplication and fragmentation of the elastic fibres.

Subendothelial humps were present in 13% of all vessels tabulated in the alcoholic group, and in 3% of the vessels in the control group. The humps consisted of a subendothelial accumulation of brightly eosinophilic, PAS positive material with a granular and occasionally hyalinized appearance. The humps were generally acellular, although mononuclear cells and cell fragments were infrequently noted within the eosinophilic material. The humps protruded in to the vascular lumens and ranged in size from 10 to 25 microns. Only the larger humps occurring in the smaller vessels appeared to significantly narrow the vascular lumen. In the rare vessels that had more than one hump, however, there appeared to be marked narrowing and distortion of the vascular channel.

The most unusual and unexpected finding in this study was the observation of Anitschkow myocytes and rarely other inflammatory cells within the intima and media of 11% of the vessels examined in the alcoholic group, and 2% of the vessels in the control group. None of these 18 cases had gross or microscopic evidence of rheumatic heart disease, nor did there appear to be increased numbers of Anitschkow cells in the interstitium. No fibrinoid necrosis or heavy inflammatory infiltrate was identified in association with these cells. Mononuclear cells with indented nuclei and a rim of cytoplasm resembling circulating monocytes were rarely noted sticking to the endothelium. The significance of these circulating cells in relation to the presence of Anitschkow myocytes within the vessel walls could not be determined.

Myofibre atrophy and variability in size, associated with oedema, fibrosis and chronic inflammation of the interstitium were noted. The methods used in this study have produced a quantitative index of vascular abnormalities in both the alcoholic and

control groups. Since qualitative abnormalities were not tabulated, it is difficult to compare the raw figures from one group to the other.

As a subjective observation, however, the vascular abnormalities in the control group, with one exception, did not approach the severity of the changes described in the alcoholic group. Endothelial disruption, large subendothelial spaces with loosening of the vessel wall layers, marked vascular sclerosis, and onion-skin-like perivascular fibrosis were not commonly noted in the control group. The differences between alcoholic and control groups were not statistically analysed because of the small sample population. However, comparison of the data reveals a two-to three-fold increase in the incidence of major vascular abnormalities with the overall trend suggests a definitely increased incidence of damaged vessels in the alcoholic heart.

1.12 Alcoholic Cardiomyopathy

There is much circumstantial evidence in humans for the development of progressive cardiac dysfunction and ultimately cardiomyopathy with long-standing excessive alcohol consumption (Moushmoush and Mansour, 1991). Chronic heavy use of alcohol is uncovered in a significant proportion of patients with unexplained myocardial disease (Alexander, 1966; Komajda et al, 1986). Necropsy studies show that cardiomyopathy is much more common among alcoholics than among non-alcoholics (Schenk and Cohen, 1970). Alcoholics even without symptoms or signs of cardiac disease commonly have abnormalities in left ventricular function, suggesting myocardial disease (Regan et al, 1969; Ahmed et al, 1980). Left ventricular filling impairment in asymptomatic chronic alcoholics may in fact be the earliest functional sign of preclinical alcoholic cardiomyopathy (Kupari et al, 1990). Alcoholic cardiomyopathy should be considered in long-standing alcoholic patient presenting with cardiomegaly or congestive heart failure with no other evident cause for heart failure. Alcoholic cardiomyopathy may occur in well-nourished persons. The disease does not respond to thiamine therapy and in some cases of short duration, it resolves after prolonged bed rest and abstinence from alcohol (Burch and Walsh, 1965; McDonald et al, 1971; Schwartz et al, 1975; Mølgaard et al, 1990; Jacob et al, 1991).

The precise quantity and duration of alcohol exposure required to produce cardiomyopathy are difficult to assess with any confidence. Nevertheless, it has been

suggested that at least 10 years of daily intake exceeding 100 to 150 gm of ethanol is required to produce clinically overt congestive heart failure (Schenk and Cohen, 1970; Goodwin, 1974).

Alcoholics with ethanol-related heart disease are generally affected in one of the three ways. First, those alcoholics who consume large quantities of carbohydrates such as beer, use their limited supplies of thiamine and develop the classic features of beri-beri cardiomyopathy. A second group presents with arrhythmias, especially atrial fibrillation. A third group presents with congestive cardiomyopathy with four-chamber enlargement and no other apparent cause of myocardial disease. Any patient with unexplained, biventricular congestive heart failure should be questioned concerning alcoholic intake and nutrition. Patients who admit to a large ethanol intake have usually been severely alcoholic for at least 10 years (Brigden and Robinson, 1964).

Patients with active alcoholic cardiomyopathy usually do not show elevations of creatine kinase of cardiac origin, the MB isoenzyme (Lederer and Gerstbrein, 1976). As in any cardiomyopathy, the disease appears or becomes exacerbated due to increase workload on the heart. The first onset of congestive failure is more apt to occur in patients with sustained hypertension, in those who find it necessary to undertake strenuous physical exertion, or in patients strained by a coincidental illness that places unusual demands on the heart. Mixed forms of congestive cardiomyopathy in alcoholics also occur because other harmful influences on the myocardial cell, such as hyperthyroidism or uncontrolled diabetes mellitus, protein-calorie malnutrition, mixed vitamin deficiency states, infectious disease, or even associated heart disease due to other causes can potentiate myocardial cell injury induced by ethanol. Simultaneous divalent ion deficiencies, especially of phosphorus, may also play important roles in the pathogenesis of this disease. The clinical picture cannot be distinguished from primary or secondary forms of dilated cardiomyopathy (Fink and Rosalki, 1979). This similarity also holds for echocardiographic features (Mathews et al, 1981) and findings at cardiac catheterization (Ahmed et al, 1980), but careful study of endomyocardial biopsy (Alexander, 1966; Bullock et al, 1969; Baandrup et al, 1981 and 1981) including measurement of myocardial enzyme activities may help to differentiate between alcoholic and idiopathic cardiomyopathies. Unfortunately, an animal model in which overt congestive cardiomyopathy occurs with chronic ethanol ingestion similar to that seen in humans has not been developed (Knochel, 1983).

Autopsy results of patients with cardiomyopathy show four-chamber enlargement, mild to moderate hypertrophy, mural thrombi are common, but coronary arteries are usually patent. Microscopically, there is patchy fibrosis, evidence of chronic inflammation, interstitial oedema, myocytolysis and small areas of necrosis (Alexander, 1975; Edmondson, 1980; Steinberg and Hayden, 1981) Examination of myocardial tissue by electron microscopy shows enlargement of mitochondria, dilatation of sarcoplasmic reticulum, increased fat deposition, and abnormalities of intercalated discs (Alexander, 1967; Edmondson, 1980; Rubin, 1981; Tsiplenkova et al, 1986). These findings are also identical to those observed in non-alcoholic dilated cardiomyopathy.

The natural course of alcoholic cardiomyopathy is relatively poor; over 40% of such patients died with an average time of 3 years in one follow up study (Demakis et al, 1974). The therapeutic role of abstinence needs emphasis because 61 to 73% of abstaining patients were clinically improved in contrast with only 10 to 13% of those who continued drinking (Demakis et al, 1974; Gunner et al, 1975). The potential reversibility of human alcoholic heart muscle disease has been shown also in carefully documented case histories and in studies using repeated endomyocardial biopsies after cessation of drinking (Bullock et al, 1970; Schwartz et al, 1975; Kupari, 1984). A less benign prognosis has been associated with lon-standing disease, suggesting that at a certain stage of the disease, the pathogenetic mechanisms may continue despite abstinence from alcohol (Gunner et al, 1975).

1.13 Haemodynamic Effects of Ethanol

The principal response of the heart to acute ethanol administration is decreased contractility (Regan et al, 1966). Ethanol's depressive effects are exaggerated by beta blockade, atropine (Child et al, 1979), and decreased venous return (Nixon et al, 1979). Blood ethanol levels of 200 mg/dl (or 43 mmol/l) or more may depress the normal response of the heart to exercise. In chronic alcoholics without clinical evidence of heart disease, a blood ethanol level of 150 mg/dl causes elevated left ventricular end diastolic pressure and decreased stroke volume (Regan et al, 1969). The cardio-depressive effects of ethanol appear to be immediately reversible after its removal by haemodialysis (Symbas et al, 1972). Dogs with cardiomyopathy induced by chronic ethanol feeding (Regan et al, 1966 and 1974) also show impaired

myocardial performance. Under general anaesthesia, these dogs showed a higher end diastolic pressure than normal, and did not increase their stroke output in response to angiotensin infusion. Saline infusion caused an appreciably greater increase of left ventricular end diastolic pressure in alcoholic dogs than in normal animals. The latter evidence suggested that chronic ingestion of ethanol in the experimental animal impairs cardiac reserve presumably by decreasing contractility. Persons with chronic alcoholism and dogs administered alcohol chronically become phosphorus deficient (Knochel, 1977; Fuller et al, 1978; Anderson et al, 1980; Blachley et al, 1980). The findings for alcoholic cardiomyopathy resemble the haemodynamic effects of chronic phosphorus deficiency in dogs (Fuller et al, 1978).

1.14 Effect of Ethanol on the Peripheral Circulation

As a result of ethanol's direct action on vascular smooth muscle cells, it may act as a vasodilator and cause hypotension (Altura and Altura, 1982). Two mechanisms have been proposed to explain the vasodilator effect: inhibition of the normal rhythm or vasomotion of vascular smooth muscle; and depression of the contractile responses to endogenous neurohumoral substances that play a role in maintaining vascular tone and regulation of blood flow.

Considerable information shows that ethanol may interfere with factors regulating calcium concentration inside smooth muscle cells or affect translocation of calcium across vascular membranes, directly interfering with contractile processes. It is not necessary to implicate either acetaldehyde or acetate for this response to occur (Puszkin and Rubin, 1975; Rubin, 1979).

Ethanol has also been implicated as a factor potentially mediating hypertension. It is noteworthy that 30% to 60% persons with long standing alcoholism have hypertensive vascular disease (Beevers, 1977; Klatsky et al, 1977; Saunders et al, 1979; Harburg, 1980). Low blood concentrations of ethanol potentiate the action of vasopressin and catecholamines (Talesnik et al, 1980) where as higher concentrations of ethanol reduce contractions induce by this neurohumoral substances (Edgarian and Altura, 1976).

Recent studies on isolated cerebral vessels from the dog show that ethanol alone can evoke potent contractions. This response is attentuated by a low calcium concentration in the fluid surrounding the artery. Any process permitting calcium concentration to rise in the cytoplasm in the muscle cell will both initiate and potentiate its contractile responsiveness. In normal rats, a 10% ethanol infusion causes vasodilatation and essentially obviates the local vasoconstrictor effects of norepinephrine when the latter is applied directly to a splanchnic vessel. In contrast, if the same experiment is done in a chronic alcoholic rat, vasodilatation does not occur and the local vasoconstrictor effects of norepinephrine are potentiated. Presumably this action results from increased movement of calcium from extracellular fluid to vascular smooth muscle cells due to increased mobility of calcium. Thus, as the cytosolic calcium concentration rises, the contractile response to circulating vasoconstrictors such as catecholamines, angiotensin II, or vasopressin, becomes accentuated (Altura and Altura, 1982) .

These studies have been extended to explain the common occurrence of transient hypertension during hypomagnesemia in the patient withdrawing from alcohol (Turlapaty and Altura, 1980). It is known that magnesium deficiency causes accumulation of calcium in various tissues, including vascular smooth muscle. As calcium ions accumulate, the contractile response to either norepinephrine or angiotensin-II becomes more pronounced. In contrast, it is probable that the vasodilatory effects of infused magnesium salts are the result of its tendency to block access of calcium to intracellular locations (Cronin et al, 1982) .

1.15 Objectives of the Study

This review of the literature tends to suggest that significant morphological features should be identifiable in the heart of persons who have been exposed over several years to excessive alcohol.

The intention of this study is to identify such changes in hearts which show no other pathology and also have not acquired features of full blown alcohol induced cardiomyopathy. Most of the publications on autopsy specimens performed on human subjects have tended to be rather subjective and descriptive. To overcome this problem a semiautomated computerised morphometric system was employed. It was further intended to correlate any morphological changes in muscle fibres and any replacement of the myocardium by fibro-connective tissue with concomitant changes within the intra-myocardial arterioles and thus also asses the hypothesis that any myocardial changes are secondary to microvascular problems which could also be related to alcohol abuse.

Chap 2

General Materials and Methods

Introduction

This study was carried out on human hearts removed at autopsy carried out as part of the forensic pathological investigation of sudden and unexpected death of the decedent.

2.1 Selection of Specimens

The hearts which formed the basis of this study can be subdivided into two groups- "the study group" from decedents known to be chronic alcohol abusers and "the control group" from non-alcohol abusers.

The hearts, both alcoholic and control groups, were collected randomly for this study from deceased persons dying suddenly and unexpectedly referred for forensic autopsy carried out in the city mortuary at Edinburgh, Bangour General Hospital, St. John's Hospital and the Borders General Hospital during the years 1991, 1992 and 1993.

For the hearts in the "control group" the following criteria had to be satisfied:
1. No evidence in the medical history available i.e. as obtained by the police and by the Procurator Fiscal by interviewing the general practitioner and other persons who knew the deceased during life, and in the general practitioner's case notes, that the deceased was a chronic heavy smoker or suffered from systemic hypertension, respiratory (e.g. COAD) or cardiac problems.
2. No history of chronic alcohol abuse was known to the persons interviewed by the police, and to the general practitioner. The general practitioner was also able to exclude the presence of any complaints of illness related to alcohol abuse or the presence of any liver damage induced by alcohol. Evidence was also obtained circumstantially from the examination of the abode of the deceased by the police,

from social workers' reports (when available) and from relatives or friends whom the police interviewed.

3. At autopsy there was no evidence on inspection of tobacco smoke staining on the fingers and excessive carbon pigmentation of the lungs.

4. The heart on inspection showed no gross pericardial, myocardial, endocardial or valvular abnormalities.

5. Sectioning of the coronary arteries or careful examination of the major peripheral arterial branches showed no stenosis in excess of 30% (due to atheroma).

6. At autopsy and subsequent extensive histological examination of all the organs, no pathology which would have had a bearing on heart changes was identified.

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1. No evidence in the medical history available i.e. as obtained by the police and by the Procurator Fiscal by interviewing the general practitioner and other persons who knew the deceased during life, and in the general practitioner's case notes that the deceased was a chronic heavy smoker or suffered from systemic hypertension, respiratory (e.g. COAD) or cardiac problems.

2. The diagnosis of chronic alcohol abuse in each case was based on the police "sudden death report" which was sent under confidential cover, in writing, to the Procurator Fiscal, and on the medical history appended to the police report. The medical history had been obtained by the police and by the Procurator Fiscal by interviewing the family medical practitioner and other persons who knew the deceased during life. The general practitioner was also able to exclude the presence of any complaints of illness related to alcohol abuse or the presence of any liver damage induced by alcohol. Evidence was also obtained circumstantially from the examination of the abode of the deceased by the police, from social workers' reports (when available) and from relatives or friends whom police interviewed.

3. At autopsy there was no evidence on inspection of tobacco smoke stains on the fingers and excessive carbon pigmentation of the lungs.

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5. Sectioning of the coronary arteries or careful examination of the major peripheral arterial branches showed no stenosis in excess of 30% (due to atheroma).

6. At autopsy and subsequent extensive histological examination of all the organs, no pathology which would have had a bearing on heart changes was identified.

All the autopsies were carried out by a consultant forensic pathologist and in all cases histology was part of this examination.

Cases of macroscopically evident cardiomyopathy with such obvious features as a combination of marked enlargement of the heart associated with marked multi-chamber dilatation with or without mural thrombus formation, a pale and flabby myocardium with a variegated appearance suggestive of focal fibrosis, were excluded.

The rationale for the exclusions in the control group was to obtain a representative cohort of patients from the same geographical and ethnic background which as much as possible was age and sex-matched to the study group. Given the spectrum of the selection - and exclusion - criteria the numbers in the control group was low, however statistically amenable to coming to the appropriate statistical analysis. Obviously there was no control on the subjects which were referred for autopsy and this is reflected in the distribution in terms of gender and age group in the "control group". This was particularly difficult because of the high incidence of coronary atheromatous changes in the Scottish population and this condition being a common cause of sudden unexpected death.
The scope of picking these controls was to ensure that the "Lothian" cases were similar in terms of body parameters and heart weights to previously published series and these specimens were treated identically to the study group to provide some control for artefactual changes.

The rationale of choosing the specific cases of chronic alcoholics selected was to help ensure that any cardiac findings which could be identified would not be the consequence of some other condition entirely unrelated to chronic alcohol abuse i.e. that any of the changes found on detailed morphometric examination were the direct effects of the alcohol abuse and not of other pathologies. It was also the intention to pick up the earlier changes of alcohol-induced damage and for this reason very abnormal hearts were excluded.

The choice of controls was limited in relation to gender match but was more balanced regards to age matching: this situation could not be improved on because the criteria used for selection and exclusion.

Collection of Specimens: In each case, both study group and the controls, a full autopsy was carried out by a consultant forensic pathologist. All the body cavities were examined, and toxicological studies carried out where indicated, including an assay of alcohol in the blood, urine and vitreous fluid. Specimens from all organs were subjected to histological examination. Once the heart was removed, the coronary arteries were examined carefully by transverse sequential cuts at 2 - 5 mm intervals. If any of the main coronary arteries or major peripheral arterial branches showed the presence of stenosis in excess of 30% due to atheroma, the heart concerned was excluded from the study. The main vessels of the heart were trimmed away close to the transverse pericardial sinus. The heart was then opened in a transverse circumferential method. In this method (see figure 2.1.1) the heart in its entirety was cut at 1cm intervals.

Any heart showing any evidence on inspection of pericarditis, valvular, ischaemic or hypertensive changes was discarded. In those cases in which subsequent autopsy histology revealed any non-alcohol related cardiac abnormality, the heart was also excluded from inclusion in the study. In none of these decedents was there any known past medical history of systemic hypertension or heart disease such as heart failure, arrhythmias, angina, etc. The smoking history of the deceased was often not available; however no cases which showed evidence of tobacco smoke stains on fingers, chronic obstructive airway disease (COAD), with or without corpulmonale, were selected. An adequate cause of death was found in all these cases and no obvious feature of cardiomyopathy was present.

"Control" hearts were also obtained from autopsies in which the death was not due to a cardiac cause and in which there was no known history, autopsy evidence or histological findings suggestive of alcohol abuse. In none of these decedents was there any known past medical history of systemic hypertension or heart disease such as heart failure, arrhythmias, angina, etc. Any heart showing any evidence on inspection of pericarditis, valvular, ischaemic or hypertensive changes was discarded. The smoking history of the deceased was often not available; however no cases which showed evidence of tobacco smoke stains on fingers, chronic obstructive airway disease (COAD), with or without corpulmonale, were selected. The hearts both from alcoholic group and controls were fixed, dissected and examined in an identical manner.

Sample Size: The hearts of all the subjects (40 alcoholics and 28 controls) which fulfilled the selection criteria set out were studied: it was not possible in the time available to find other hearts of similar qualities. This gave numbers in excess of the minimum of the twenty per group, recommended on statistical grounds by an expert medical statistician, to give high enough power of detecting differences in means between groups of approximately one standard deviation.

The demographic details of the control group are shown in the table 2.1.1 and the causes of death in the control group are shown in the table 2.1.2.

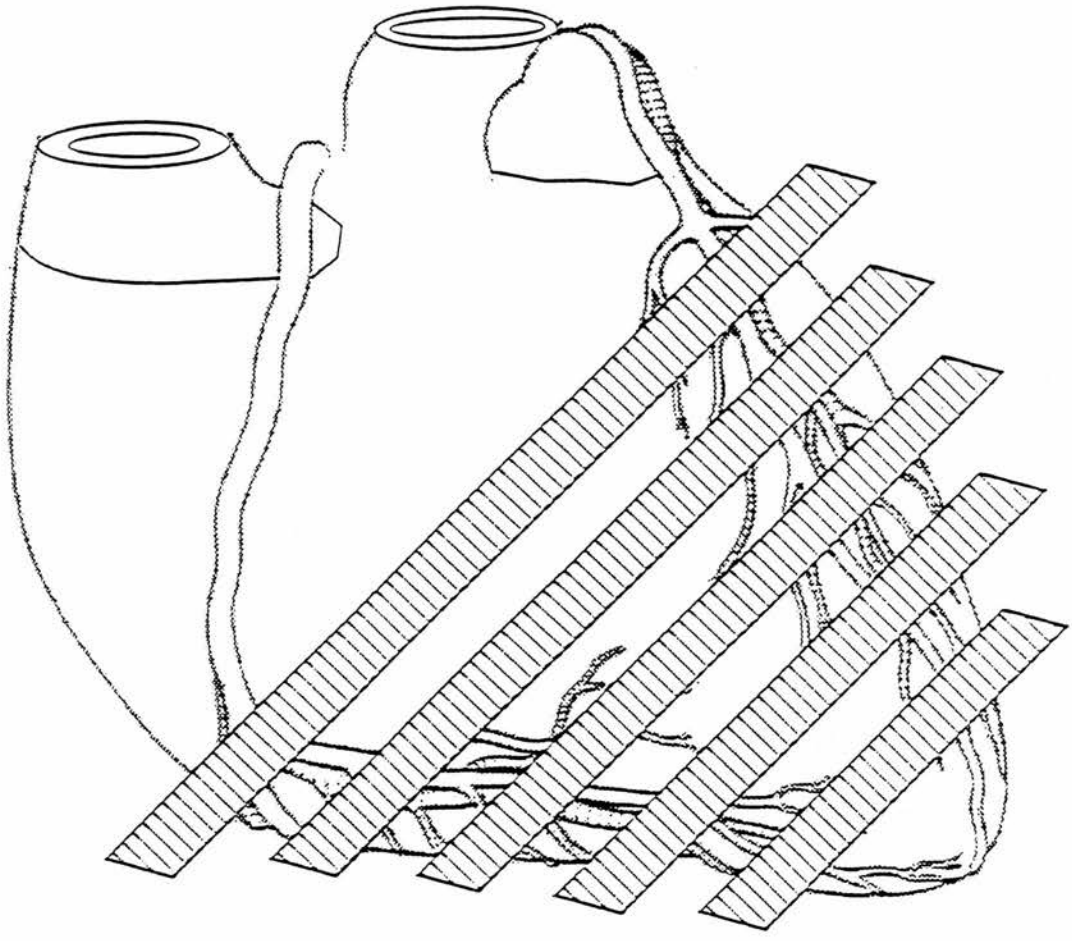


Figure 2.1.1 Transverse circumferential method of dissecting the heart at autopsy.

Table 2.1.1: Demographic details of the 'Control group'.

Case no.	Age (years)	Gender	Occupation	Body Height (cm)	Body Weight (kg)	Body Mass Index
1	41	F	Housewife	160	65	25
2	36	M	Professional	165	57	20
3	81	M	Retired	165	68	24
4	29	M	Professional	183	79	23
5	73	M	Retired	155	35	14
6	60	F	Retired	168	63	22
7	36	M	Manual	170	103	35
8	32	M	Professional	173	75	25
9	29	F	Housewife	175	106	34
10	39	M	Manual	178	75	23
11	91	M	Retired	173	60	20
12	77	M	Retired	173	76	25
13	54	M	Clerical	170	55	19
14	17	M	Manual	183	60	17
15	19	M	Professional	168	57	20
16	46	M	Manual	178	84	26
17	70	M	Retired	168	63	22
18	20	F	Professional	163	53	19
19	22	M	Professional	185	67	19
20	22	M	Manual	183	83	24

Table 2.1.1 continued

Case no.	Age (years)	Gender	Occupation	Body Height (cm)	Body Weight (kg)	Body Mass Index
21	60	M	Retired	173	68	22
22	43	M	Professional	173	67	22
23	46	M	Clerical	183	95	28
24	37	M	Unemployed	183	80	23
25	50	M	Manual	178	103	32
26	43	M	Manual	178	71	22
27	24	M	Manual	170	79	27
28	67	M	Manual	173	63	21

Table 2.1.2: Causes of death in the 'Control group'.

Case no.	Age (years)	Gender	Causes of death
1	41	F	Multiple injuries due to RTA
2	36	M	Incised wounds to neck
3	81	M	Ruptured aortic aneurysm
4	29	M	Status epilepticus
5	73	M	Hypothermia
6	60	F	Multiple injuries due to RTA
7	36	M	Crushing injury to the chest due to an industrial accident
8	32	M	Inhalation of volatile hydrocarbons
9	29	F	Acute pulmonary thrombo-embolism Pelvic vein thrombosis
10	39	M	Carbon monoxide poisoning due to inhalation of vehicle exhaust fumes
11	91	M	Acute cerebral infarct due to left carotid artery thrombosis
12	77	M	Multiple injuries due to RTA
13	54	M	Massive haemoptysis due to invasive bronchogenic carcinoma
14	17	M	Multiple injuries due to RTA

Table 2.1.2 continued

Case no.	Age (Years)	Gender	Causes of death
15	19	M	Multiple injuries due to RTA
16	46	M	Multiple injuries due to RTA
17	70	M	Multiple injuries due to fall from a height
18	20	F	Multiple injuries due to RTA
19	22	M	Multiple injuries due to RTA
20	22	M	Multiple injuries due to an industrial accident
21	60	M	Acute epileptic attack
22	43	M	Amitriptyline overdose Mental depression
23	46	M	Electric shock
24	37	M	Acute pulmonary oedema Ventricular arrhythmia
25	50	M	Pulmonary embolism due to peripheral venous thrombosis
26	43	M	Multiple injuries due to RTA
27	24	M	Multiple injuries due to RTA
28	67	M	Pulmonary embolism due to phlebothrombosis of both calves

2.1.2 Actual Specimens Collected

Hearts from 40 deceased Caucasians known to suffer from chronic alcoholism, in whom several years excessive and recurrent consumption of alcohol was well documented, formed the basis of this study.

The demographic details of the chronic alcoholic group are shown in the table 2.1.3 and the causes of death in the chronic alcoholic group are shown in the table 2.1.4.

2.1.3 Background Information on Cases

In each case a detailed report had been submitted to the Procurator Fiscal by the police after a full investigation by a specially trained police enquiry officer. The investigation included the full circumstances of the death and the known past medical and social history of the deceased.

Other data which was found in the final report made to the Procurator Fiscal (who is the legal authority that investigates sudden death in Scotland) are the following:

(1) Demographic details- age, sex, marital status, occupation, height, weight, etc.
(2) Past medical history of the deceased, including hospitalisation and medical complaints.
(3) Any current medication.

For those who died in hospital, the hospital notes were also available.

Table 2.1.3: Demographic details of the 'Alcoholic group'.

Case no.	Age (Years)	Gender	Occupation	Body Height (cm)	Body Weight (kg)	Body Mass Index
1	54	M	Manual	179	63	19
2	62	M	Manual	188	68	19
3	39	F	Manual	152	53	22
4	62	F	Manual	150	50	22
5	30	F	Unemployed	163	65	24
6	56	M	Clerical	173	68	22
7	49	F	Clerical	160	77	30
8	66	F	Retired	155	36	14
9	71	M	Retired	168	54	19
10	45	M	Unemployed	183	80	23
11	47	F	Manual	160	54	21
12	47	M	Manual	173	102	34
13	46	F	Housewife	160	61	23
14	40	F	Housewife	155	60	24
15	44	F	Housewife	152	46	19
16	30	M	Unemployed	160	51	19
17	27	M	Unemployed	177	64	20
18	50	F	Housewife	163	60	22
19	45	M	Professional	175	78	25
20	59	F	Retired	160	43	16

Table 2.1.3 continued

Case no.	Age (Years)	Gender	Occupation	Body Height (cm)	Body Weight (kg)	Body Mass Index
21	51	M	Manual	160	58	22
22	65	M	Retired	178	82	25
23	47	F	Housewife	173	64	21
24	29	M	Professional	175	60	19
25	43	M	Unemployed	175	91	29
26	54	M	Professional	168	70	24
27	51	F	Unemployed	173	65	21
28	51	M	Unemployed	165	87	31
29	65	F	Retired	147	44	20
30	57	M	Unemployed	160	49	19
31	47	M	Unemployed	185	64	18
32	33	F	Housewife	155	65	27
33	32	F	Clerical	168	63	22
34	57	M	Unemployed	170	83	28
35	42	F	Manual	152	65	28
36	65	M	Retired	175	94	30
37	43	M	Unemployed	170	84	29
38	60	M	Manual	170	58	20
39	52	M	Unemployed	160	39	15
40	59	F	Housewife	163	70	26

Table 2.1.4: Causes of death in the 'Alcoholic group'.

Case no.	Age (Years)	Gender	Causes of death
1	54	M	Acute pulmonary oedema Status epilepticus
2	62	M	Acute pulmonary thrombo-embolism due to pelvic and deep vein thrombosis
3	39	F	Dextropropoxyphene overdose
4	62	F	Amitriptyline and alcohol overdose
5	30	F	Acute on chronic alcoholism
6	56	M	Acute on chronic alcoholism
7	49	F	Subdural haematoma due to Von Willebrands' disease
8	66	F	Acute pancreatitis Fatty liver
9	71	M	Acute on chronic alcoholism
10	45	M	Multiple injuries due to RTA
11	47	F	Alcohol and diazepam overdose
12	47	M	Hepatic failure due to alcoholic cirrhosis of the liver
13	46	F	Acute on chronic alcoholism

Table 2.1.4 continued

Case no.	Age (Years)	Gender	Causes of death
14	40	F	Epileptic fit from an alcohol withdrawal syndrome
15	44	F	Smoke inhalation and burns in a house fire
16	30	M	Acute on Chronic alcoholism
17	27	M	Acute paracetamol poisoning
18	50	F	Early basal pneumonia Acute on chronic alcoholism
19	45	M	Extensive haemorrhage due to epistaxis
20	59	F	Inhalation of fire fumes in a house fire
21	51	M	Inhalation of gastric contents Acute on chronic alcoholism
22	65	M	Multiple injuries due to a fall from a height
23	47	F	Acute Amitriptyline poisoning
24	29	M	Acute carbon monoxide poisoning due to inhalation of car exhaust fumes
25	43	M	Hepatic cirrhosis Acute on chronic alcoholism
26	54	M	Hanging

Table 2.1.4 continued

Case no.	Age (Years)	Gender	Causes of death
27	51	F	Acute on chronic alcoholism
28	51	M	Acute haemorrhagic pancreatitis Acute on chronic alcoholism
29	65	F	Status epilepticus
30	57	M	Fatty infiltration of the myocardium
31	47	M	Fracture of the cervical spine due to hanging
32	33	F	Acute on chronic alcoholism
33	32	F	Ruptured oesophageal varices due to hepatic cirrhosis
34	57	M	Acute paracetamol and alcohol overdose
35	42	F	Acute lobar pneumonia Hepatic cirrhosis
36	65	M	Multiple injuries due to RTA
37	43	M	Dextropropoxyphene and alcohol overdose
38	60	M	Acute on chronic alcoholism
39	52	M	Bronchopneumonia Carcinoma of the hypopharynx
40	59	F	Acute Amitriptyline overdose

Table 2.1.5: Summary of causes of death in alcoholic cases chosen for this study.

Causes of death	**No. of cases**	**Percentage**
Acute on chronic alcoholism	8	20
Combined alcohol & drug overdose	4	10
Acute drug overdose	4	10
Traumatic	5	12.5
Complication of hepatic cirrhosis	3	7.5
Pneumonia	3	7.5
Pancreatitis	2	5
Epilepsy	2	5
House fire	2	5
Other causes*	7	17.5

*(acute pulmonary oedema; pulmonary thrombo-embolism; subdural haematoma; extensive haemorrhage; inhalation of gastric contents; carbon monoxide poisoning; fatty infiltration of the myocardium)

2.1.4 Body Mass Index (BMI)

This was calculated according to the formula:

$$\text{Body Mass Index (BMI)} = \text{Weight in (kg)} / \text{Height in (meter)}^2$$

This is a standard physiological parameter and will be used in a number of the analyses that were carried out. This index corrects for such variables as the weight and height of the deceased which directly influence the weight of the internal organs.

2.2 Heart Weights

The weight of the fresh heart specimen was obtained in every case at autopsy. After fixation for variable periods over two weeks in 10% neutral buffered formalin, the heart weight was obtained once again (see table 2.2.1 and table 2.2.2). In both instances an electronic balance was used and the organ weighed to the nearest gram after blotting with dry paper towel.

2.3 Fixation of the Hearts

Most tissues are fixed before they are examined microscopically. Fixation is the foundation for the subsequent stages in the preparation of the sections. For ease of carrying out these subsequent techniques, it is essential that fixation is effective and that the appropriate fixative is used. The aims of fixation of tissues are, prevention from the process of autolysis and bacterial attack; prevention from change of shape or volume during any of the subsequent procedures and they should be left in a condition which subsequently allows clear staining of sections. At the same time, tissues should be as close to their living state as possible and, ideally, no small molecules should be lost.

Table 2.2.1: Total fresh heart weight, fixed heart weight and the Body Height, Body Weight and Body Mass Index of the 'Control group'.

Case no.	**Age (years)**	**Gender**	**Body Height (cm)**	**Body Weight (kg)**	**Body Mass Index**	**Fresh Ht. Wt. (gm)**	**Fixed Ht. Wt. (gm)**
1	41	F	160	65	25	270	232
2	36	M	165	57	20	300	287
3	81	M	165	68	24	390	360
4	29	M	183	79	23	420	395
5	73	M	155	35	14	280	264
6	60	F	168	63	22	320	286
7	36	M	170	103	35	380	350
8	32	M	173	75	25	400	381
9	29	F	175	106	34	380	365
10	39	M	178	75	23	300	290
11	91	M	173	60	20	350	321
12	77	M	173	76	25	300	290
13	54	M	170	55	19	390	370

Table 2.2.1 continued

Case no.	Age (Years)	Gender	Body Height (cm)	Body Weight (kg)	Body Mass Index	Fresh Ht. Wt. (gm)	Fixed Ht. Wt. (gm)
14	17	M	183	60	17	330	310
15	19	M	168	57	20	310	295
16	46	M	178	84	26	380	370
17	70	M	168	63	22	320	310
18	20	F	163	53	19	220	208
19	22	M	185	67	19	340	325
20	22	M	183	83	24	400	370
21	60	M	173	68	22	310	263
22	43	M	173	67	22	390	380
23	46	M	183	95	28	430	415
24	37	M	183	80	23	350	341
25	50	M	178	103	32	460	446
26	43	M	178	71	22	330	320
27	24	M	170	79	27	340	330
28	67	M	173	63	21	360	313

Table 2.2.2: Total fresh heart weight and fixed heart weight and the Body Height, Body Weight and Body Mass Index of the 'Alcoholic group'.

Case no.	**Age (Years)**	**Gender**	**Body Height (cm)**	**Body Weight (kg)**	**Body Mass Index**	**Fresh Ht. Wt. (gm)**	**Fixed Ht. Wt. (gm)**
1	54	M	179	63	19	410	390
2	62	M	188	68	19	555	530
3	39	F	152	53	22	270	261
4	62	F	150	50	22	300	290
5	30	F	163	65	24	280	269
6	56	M	173	68	22	400	384
7	49	F	160	77	30	360	351
8	66	F	155	36	14	220	210
9	71	M	168	54	19	380	352
10	45	M	183	80	23	400	361
11	47	F	160	54	21	320	303
12	47	M	173	102	34	590	560
13	46	F	160	61	23	350	342
14	40	F	155	60	24	300	283

Table 2.2.2 continued

Case no.	Age (Years)	Gender	Body Height (cm)	Body Weight (kg)	Body Mass Index	Fresh Ht. Wt. (gm)	Fixed Ht. Wt. (gm)
15	44	F	152	46	19	280	298
16	30	M	160	51	19	280	265
17	27	M	177	64	20	500	478
18	50	F	163	60	22	260	250
19	45	M	175	78	25	470	460
20	59	F	160	43	16	260	250
21	51	M	160	58	22	310	297
22	65	M	178	82	25	490	478
23	47	F	173	64	21	300	280
24	29	M	175	60	19	330	293
25	43	M	175	91	29	520	505
26	54	M	168	70	24	340	318
27	51	F	173	65	21	350	329
28	51	M	165	87	31	510	444
29	65	F	147	44	20	300	283

Table 2.2.2 continued

Case no.	Age (Years)	Gender	Body Height (cm)	Body Weight (kg)	Body Mass Index	Fresh Ht. Wt. (gm)	Fixed Ht. Wt. (gm)
30	57	M	160	49	19	320	294
31	47	M	185	64	18	310	307
32	33	F	155	65	27	290	282
33	32	F	168	63	22	250	225
34	57	M	170	83	28	440	422
35	42	F	152	65	28	490	470
36	65	M	175	94	30	570	545
37	43	M	170	84	29	400	386
38	60	M	170	58	20	380	308
39	52	M	160	39	15	260	246
40	59	F	163	70	26	410	409

All heart specimens in the alcoholic group and in the control group were treated identically to ensure that any artefacts would be represented equally throughout all the specimens.

The cross sectional specimens of these hearts were fixed flat in 10% buffered formal saline for several weeks to ensure proper fixation of the entire myocardium. A volume of formalin solution ten times that of each specimen was used in each case.

2.4 Assessment of Hypertrophy

Separate left and right ventricular weights with and without epicardial fat were obtained using a modification of the method published by Fulton et al (1952) and Lamb (1973), as follows:

The first step in each dissection was the separation of the atria and the roots of the great vessels from the ventricles by cutting along the atrio-ventricular groove using blunt-tipped scissors. Placing one blade anteriorly on the upper surface of the left ventricle and cutting laterally and posteriorly along the atrio-ventricular ring trimmed off the atria, the circumflex coronary artery and the mitral valve in one cut. The cut was continued posteriorly, enabling the scissors to turn at the left side of the heart to trim across the top of the posterior surfaces of the left ventricle and of the right ventricle following the ring. The next step was to trim along the top of the anterior wall of the right ventricle following the atrio-ventricular groove and to cut across the pulmonary artery below the valve cusps and across the base of the aorta. Then, trimming off any remaining aorta from the back of the pulmonary conus, the aortic valve was removed. Any remnants of atrio-ventricular valve tissues were trimmed off.

Holding the left ventricle in the palm of the left hand and keeping the scissors flat against the septum, the right ventricular wall was separated from the left ventricle and septum. The ridge of tissue remaining was removed by a second cut. Finally, placing the scissors flat against the right side of the septum, any muscle bands protruding into the lumen were removed and included with the right ventricle (see figure 2.4.1). Separate left ventricular weight with septum and right ventricular weight were then recorded on an electronic balance.

The epicardial fat was then trimmed away along with the coronary arteries with scissors, followed by scraping with a blunt scalpel to remove any remaining fat (see figure 2.4.2). Then, after blotting with a dry paper towel, the new weights of the right ventricle and the left ventricle with septum were recorded on an electronic balance.

Right and left ventricular weights with and without fat and the total fixed heart weights both in controls and in the alcoholic group is shown in tables 2.4.1 and 2.4.2.

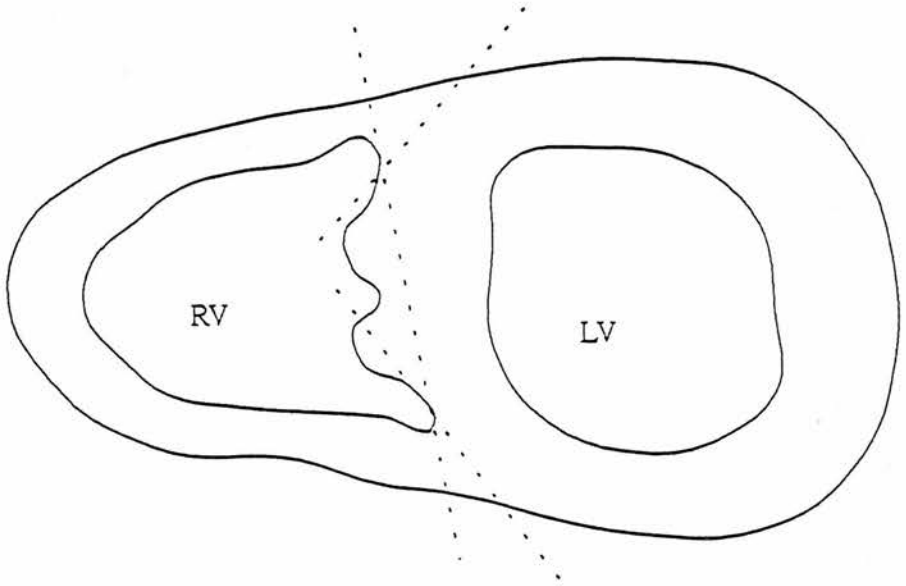


Figure 2.4.1 Separation of the right ventricle from the left ventricle.

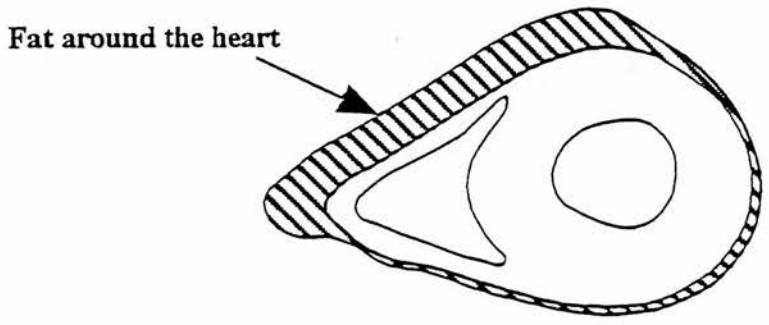


Figure 2.4.2 Diagrammatic representation of cross-section of the heart showing the distribution of epicardial fat that was trimmed off.

Table 2.4.1: Right and left ventricular weights with and without fat and total fixed heart weight in 'controls'.

Case no.	LV with fat (gm)	LV less fat (gm)	RV with fat (gm)	RV less fat (gm)	Fixed Heart wt. (gm)
1	118	110	53	37	232
2	157	149	73	57	287
3	159	139	58	42	360
4	206	176	96	60	395
5	130	115	58	34	264
6	137	112	70	49	286
7	200	180	78	58	350
8	195	185	77	52	381
9	171	159	116	86	365
10	135	124	75	52	290
11	137	124	71	50	321
12	134	126	58	38	290
13	200	177	98	69	370
14	138	117	69	43	310
15	140	129	82	51	295
16	213	194	84	59	370
17	137	116	67	42	310
18	122	114	57	41	208
19	180	162	82	56	325
20	200	176	97	68	370
21	143	137	56	48	263
22	190	170	82	62	380
23	229	178	86	55	415
24	153	130	98	56	341
25	230	206	100	70	446
26	168	150	83	69	320
27	143	128	76	55	330
28	144	130	90	63	313

Table 2.4.2: Right and left ventricular weights with and without fat and total fixed heart weight in 'alcoholics'.

Case no.	LV with fat (gm)	LV less fat (gm)	RV with fat (gm)	RV less fat (gm)	Fixed Heart wt. (gm)
1	192	159	121	73	390
2	300	263	132	91	530
3	146	132	67	52	261
4	138	125	85	63	290
5	125	116	70	56	269
6	191	177	113	87	384
7	158	128	107	56	351
8	97	87	70	56	210
9	155	145	85	63	352
10	195	181	92	66	361
11	139	130	86	64	303
12	290	250	180	97	560
13	134	111	122	67	342
14	144	114	64	36	283
15	148	126	97	53	298
16	143	132	65	51	265
17	284	250	104	72	478
18	142	131	64	49	250
19	230	212	122	89	460
20	120	92	73	39	250
21	138	117	87	65	297
22	214	180	148	75	478
23	142	112	63	48	280
24	155	136	80	54	293
25	266	241	144	103	505
26	159	149	76	60	318
27	153	142	83	64	329

Table 2.4.2 continued

Case no.	LV with fat (gm)	LV less fat (gm)	RV with fat (gm)	RV less fat (gm)	Fixed Heart wt. (gm)
28	213	183	131	88	444
29	149	128	99	65	283
30	139	128	82	51	294
31	149	129	89	58	307
32	143	113	63	35	282
33	117	106	53	41	225
34	222	191	109	76	422
35	254	210	133	100	470
36	270	230	160	87	545
37	210	180	95	62	386
38	127	104	75	49	308
39	122	110	64	44	246
40	191	158	120	58	409

2.5 Heart Blocks

After fixation for several weeks, three slices were chosen from all of the heart specimens from the proximal, middle and distal third of the heart at approximately 2 cm, 4 cm and 6 cm respectively from aortic-valbular ring (see figure 2.5.1). Blocks at standard levels from all of the heart specimens were taken and sections were collected from the left atrium, the right ventricle and from different levels of the left ventricle using a sharp scalpel and were post-fixed in 10% buffered formalin. The heart blocks chosen (see figure 2.5.2) for microscopic and morphometric study were the following:

a. Anterior wall of the Left Atrium
b. Anterior wall of the Right Ventricle (4 cm from the pulmonary valvular ring)
c. Anterior wall of the Left Ventricle (proximal third, 2 cm from the aortic valvular ring)
d. Lateral wall of the Left Ventricle (middle third, 4 cm from the aortic valvular ring)
e. Septum (middle third, 4 cm from the aortic valvular ring)
f. Posterior wall of the Left Ventricle (distal third, 6 cm from the aortic valvular ring)

At an early stage in the study it was decided that the atrial musculature, vessels and connective tissue component were markedly different from the ventricular component and as such atrial blocks were not included in the analysis.

2.6 Tissue Processing and Preparation of Slides

The aim of tissue processing is to embed the tissue in a solid medium firm enough to support the tissue and give it sufficient rigidity to enable thin sections to be cut, and yet soft enough to enable the knife to cut the sections with little damage to knife or tissue. After effective fixation in formalin for several days the tissue blocks were dehydrated to remove aqueous fixative and any tissue water with alcohol and then clearing with xylene the tissues were paraffin wax embedded and 3 - 4 μm thick sections were cut in a standard fashion with a Leitz 1512 rotary microtome, this instrument has built-in motor drive with foot or hand control.

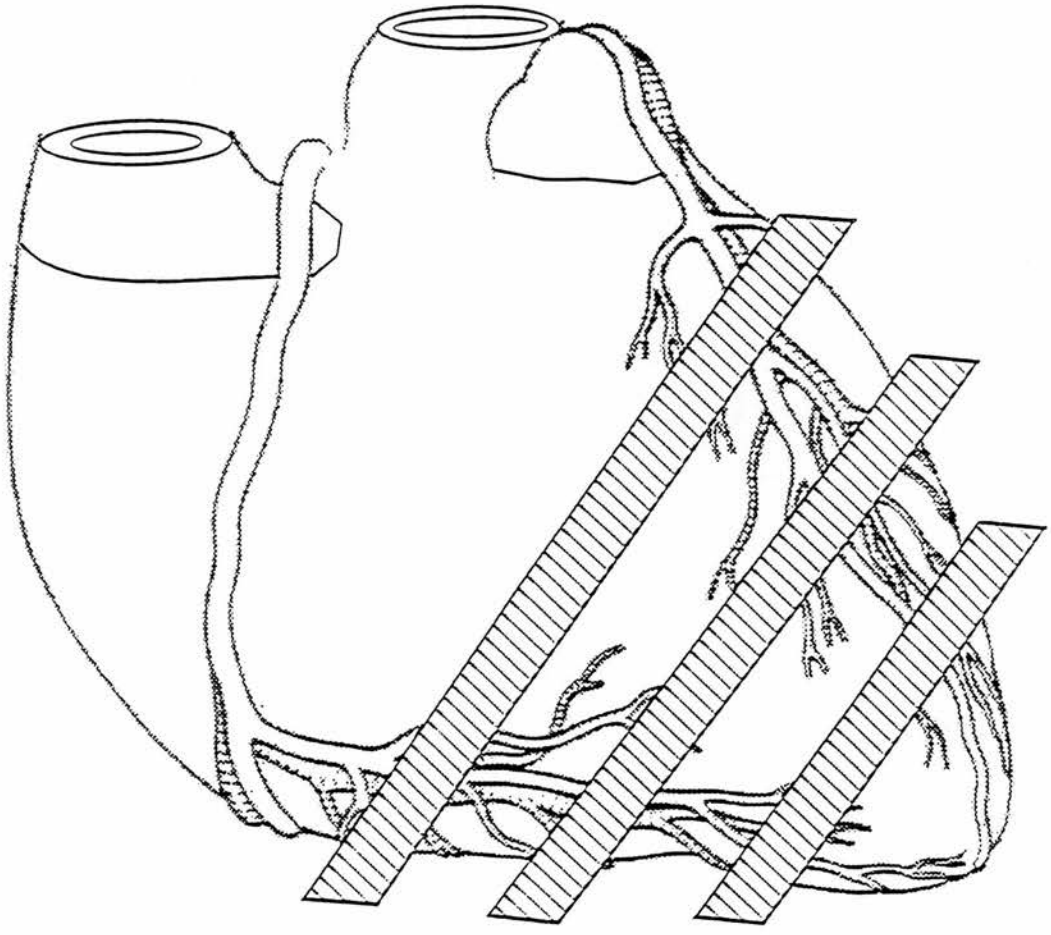
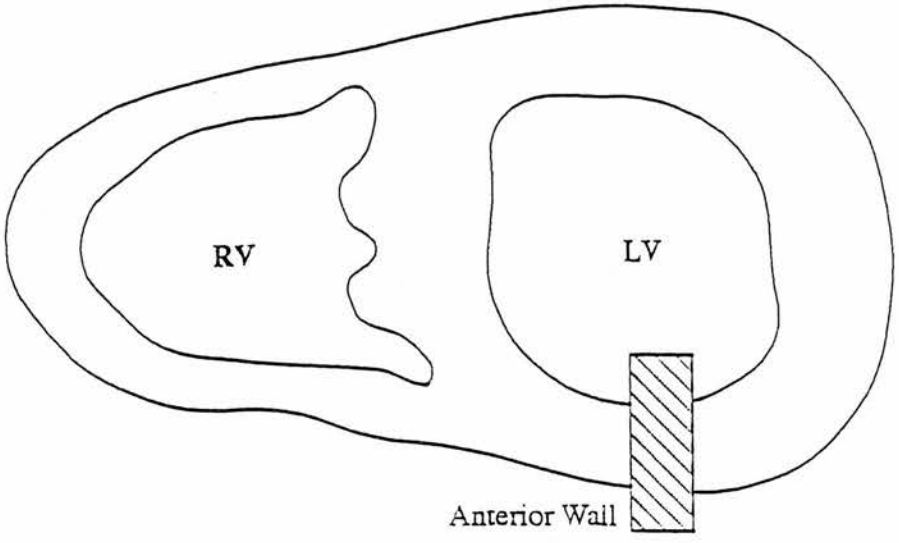
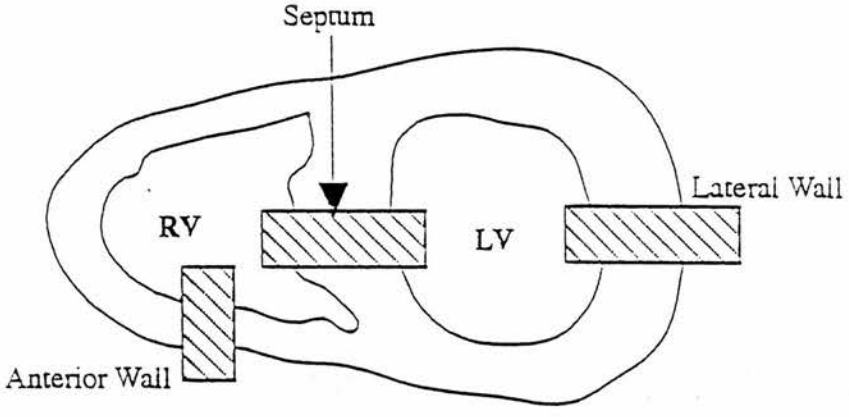


Figure 2.5.1 The manner in which gross sections were taken from different levels of heart at approximately 2 cm, 4 cm and 6 cm from the aortic valvular ring.

PROXIMAL 1/3rd OF HEART



MIDDLE 1/3rd OF HEART



DISTAL 1/3rd OF HEART

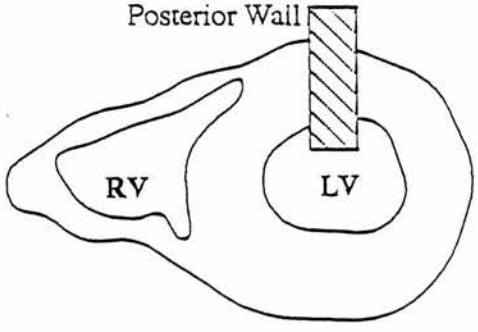


Figure 2.5.2 The regions from which the tissue blocks were selected for histology from the gross heart slices.

The slides were then stained with H&E to enable light microscopic and morphometric study of muscle fibre and connective tissue. The H&E stain is probably the most widely used histological stain. Its popularity is based on its ability to demonstrate clearly an enormous number of different tissue structures, and its comparative simplicity. Essentially, the haematoxylin component stains the cell nuclei blue-black, with good intra-nuclear detail, whilst the eosin stains cell cytoplasm and most connective tissue fibres in varying shades and intensities of pink, orange and red.

For morphometric study of the blood vessels serially obtained sections were stained additionally with Van Geison's elastic stain, and for assessment of any vascular wall infiltration with the Periodic Acid-Schiff (PAS) stain.

2.6.1 Staining of Elastic Tissue Fibres

Numerous tinctorial techniques have been evolved for the demonstration of elastic tissue fibres, although few are in current use. Of these, one of the most popular is Van Geison's elastic stain method which works after any fixative. It gives an intense black staining of the coarse fibres, but finer fibres are less well demonstrated.

The steps of staining followed in this method are:

1. Dewaxing sections and taking sections to water
2. Celestine blue for 5 minutes
3. Rinse in water
4. Haematoxylin for 4 minutes
5. Differentiating and blueing up
6. Staining with Van Geison's solution (1 part Acid Fuchsin and 14 parts saturated Picric acid) for 5 minutes
7. Brief rinse in water
8. Rapidly dehydrating with absolute alcohol, clearing in xylene and mounting

2.6.2 Periodic Acid-Schiff (PAS) Staining

The PAS reaction is a useful indicator of the presence of tissue carbohydrates. The principle of the reaction is that periodic acid will bring about oxidative cleavage of the carbon to carbon bond to form di-aldehydes. The steps of staining employed in this method are the following:

1. Dewaxing sections and taking the slides to water
2. Treated with 1% periodic acid for 5 minutes
3. Washing well with several changes of water
4. Treated with Schiff's reagent for 5-10 minutes
5. Washing well in running water for 5-10 minutes
6. Counter stain in haematoxylin for 2 minutes
7. Differentiating as appropriate in acid alcohol and blueing as usual
8. Washing in water
9. Dehydrating with absolute alcohol, clearing in xylene and mounting

Chapter 3

Morphometry

Introduction

The prime scope of this study was to compare several aspects of cardiac morphology and histology in a series of hearts removed at autopsy from decedents known to have long history of chronic alcohol misuse with controls selected from persons dying suddenly and unexpectedly and not known to have abused alcohol.

To attain the most accurate measurements possible a semi-automatic computer-assisted morphometric method was used on the sections taken from various parts of the heart which had been stained by simple tinctorial methods.

In each of the slides from the heart the area of tissue available for inspection comprised the following: muscle fibres and inter fascicular connective tissue in which the neurovascular bundles are present. In addition, however, there were spaces in which no tissue could be identified. These areas represent either sites of oedema and/or transudation of fluid, but are more likely to be due to artefact in the fixation, sectioning, dehydration, staining and mounting of the sections. As such it was quite important to exclude such "empty spaces" from all other measurements.

3.1 Variables Selected for Measurement

The following are the variables on which measurements were carried out on each of the heart slides. From each of the specimens six slides were produced i.e. one from the left atrium, one from the right ventricle and the other four from the left ventricle.

The variables to be measured were:

i. Area occupied in the stained slide by muscle fibres
ii. Area occupied by connective tissue
iii. Diameter of cardiac muscle fibres
iv. Area occupied by the nucleus within individual muscle fibres
v. Diameter of nucleus
vi. The intra-myocardial arterioles were also targeted as an additional site for measurement. For any arteriole that was rounded or oval measurements of it were made are as follows:

a. Circumferential length and diameter of the area bounded by the internal elastic lamina of arterioles (IEL)
b. Area and diameter of the lumen
c. Area and diameter of the intima
d. Area and diameter of the media
e. Circumferential length and diameter of the area bounded by the external elastic lamina of arterioles (EEL)

The adventitia merges with the adjacent perivascular connective tissue and it is not possible under the microscope to distinguish the point of transition. Therefore, adventitial measurement because of this intrinsic difficulty were not carried out.

3.2 Semi-automatic Morphometric Method

Measurement of different variables indicated were carried out on the 3-4 μm thick sections routinely stained and specially stained slides prepared from all the chosen blocks of alcoholic and control hearts. This was done semi-automatically on a multipurpose image-analysis workstation called Interactive Bild Analysen System (IBAS) [see figure 3.2.1].

Figure 3.2.1 Showing different parts of IBAS.
a= Leitz Ortholux-2 microscope; b= video camera; c= KAT image analysing system (IBM compatible computer); d= colour monitor; e= RGB monitor; f= MIAP2 image processing unit; g= digital tablet and stylus; h= keyboard; i= list printer.

Description of Interactive Bild Analysen System (IBAS):

This multipurpose image-analysis workstation consisted of a Leitz Ortholux-2 microscope, coupled to a high resolution black & white Sony CCD xc 77CE video camera. The video image was inputted to a Kontron AT (KAT) image analysis system, coupled with a Kontron visual display unit called colour monitor plus a MIAP2 image processing unit linked with a high resolution RGB monitor, a digital tablet and stylus, a keyboard and a list printer (see figure 3.2.2).

The Kontron AT consisted of a 386 KAT (IBM compatible) computer, linked to an image capture and processing unit consisting of a frame grabber board, image processing board - MIAP2 (Microprogrammable image array processor) and 16 Megabytes of video memory. All the software was controlled by the 386 KAT. All data collection and manipulation were carried out on this computer. The software used was dedicated to the IBAS workstation called IBAS version 2.0.

The image on the RGB monitor is displayed according to a scale of 3 primary colours. Careful manipulation of the colour intensity brings out into sharp contrast the items which are intended to be selectively measured enabling these to be shown as such on the attached monitor. Once this operation has been concluded the images through the link with the image processing system can be subjected to measurements as programmed for in the soft ware that has been used with 386 KAT computer. Images as apparent to the image processing unit can be viewed in the RGB monitor linked to the image processing unit, to the latter colour and/or list printer can be attached allowing the results obtained on the coloured image to be printed out. On the program monitor (colour monitor) attached to the KAT computer it is possible to select the program which is the most appropriate for the measurement task in hand. Within the image processing unit images are digitised into 512 x 512 pixels on a grey scale ranging from 0 (black) to 255 (white).

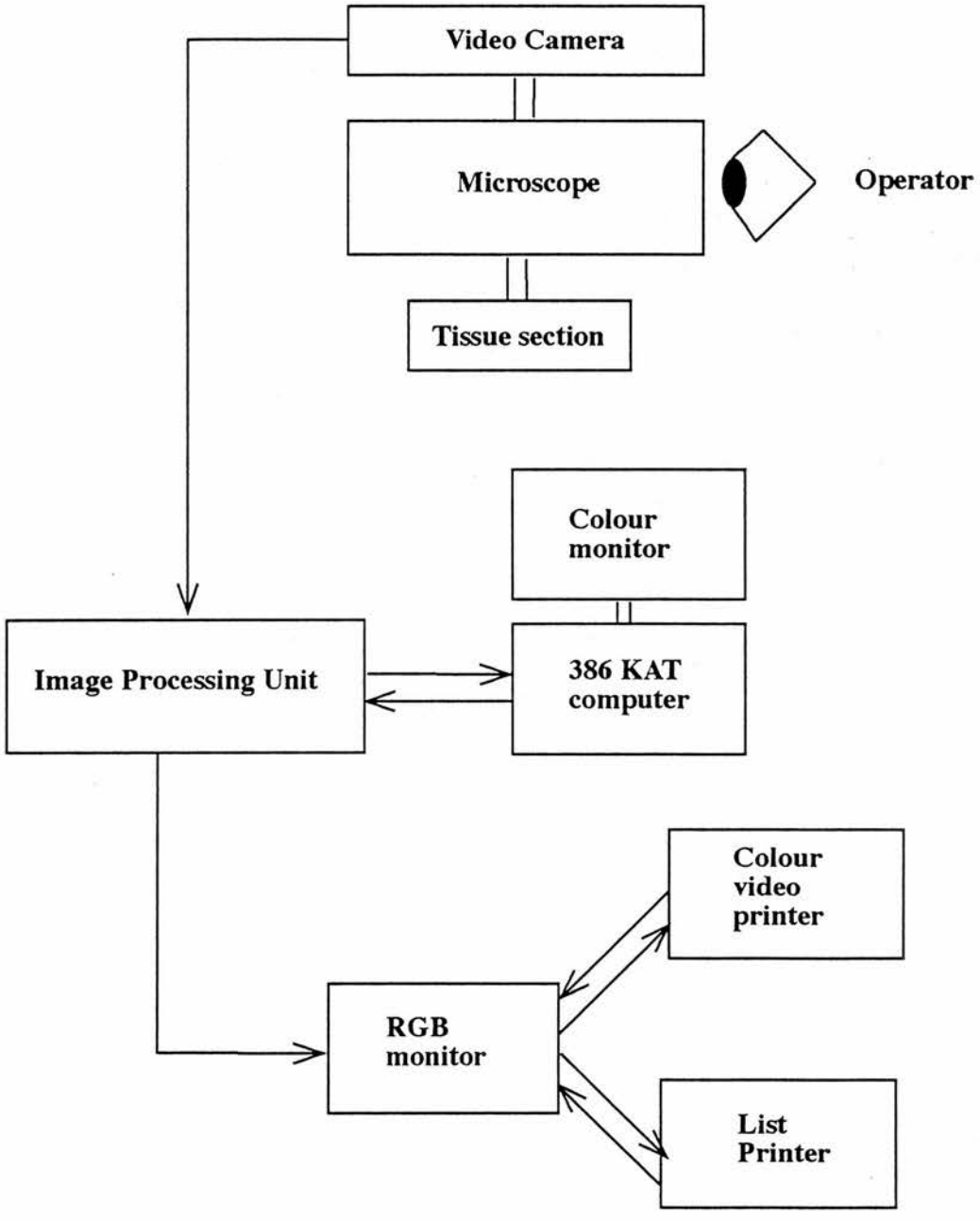


Figure 3.2.2 Diagrammatic representation of different parts of IBAS.

The software to enable these measurements to be carried out effectively was written in conjunction with Mr. D.S. Cunningham of the Imaging Unit, Pathology Department, University of Edinburgh. Three different programs i.e. one for muscle and connective tissue measurement, one for blood vessel measurement and one for nuclear measurement, were written for this specific purpose (see appendix). At the time of writing the software it was considered that fixing of the grey level to one set of values would not give the flexibility considered necessary because of variability in section thickness and staining intensity from slide to slide.

Unless specifically calibrated by the user, the IBAS workstation measures in pixel areas or pixel lengths. In order to convert measurements into microns, the software had to be calibrated using a μm calibration slide. This μm calibration slide had a 2mm line divided into 10 μm divisions (see figure 3.2.3). Using this slide, the system was calibrated in both X and Y axis directions. The system produced images of 512 x 512 pixels square.

3.3 Validation of the Semiautomatic Method

The accuracy of the computer programs has been validated in its uses in numerous projects and shown to produce more accurate results than the measurements carried out with graticules under the light microscope. Any discrepancies that could be identified between the two sets of measurements were due to the 'gating procedure' i.e. in selecting the appropriate grey level threshold values on the grey scale of the machine or marking the exact site where the light pen is applied to measure the diameter of muscle fibre or tracing around external elastic lamina of blood vessel.

The accuracy of the program, as compared to that of a human operator taking measurements with graticules under the light microscope, was considered always to be significantly better, particularly over any substantial period of time, as a human operator would invariably be subject to both fatigue and tedium, unlike the machine-operated program system.

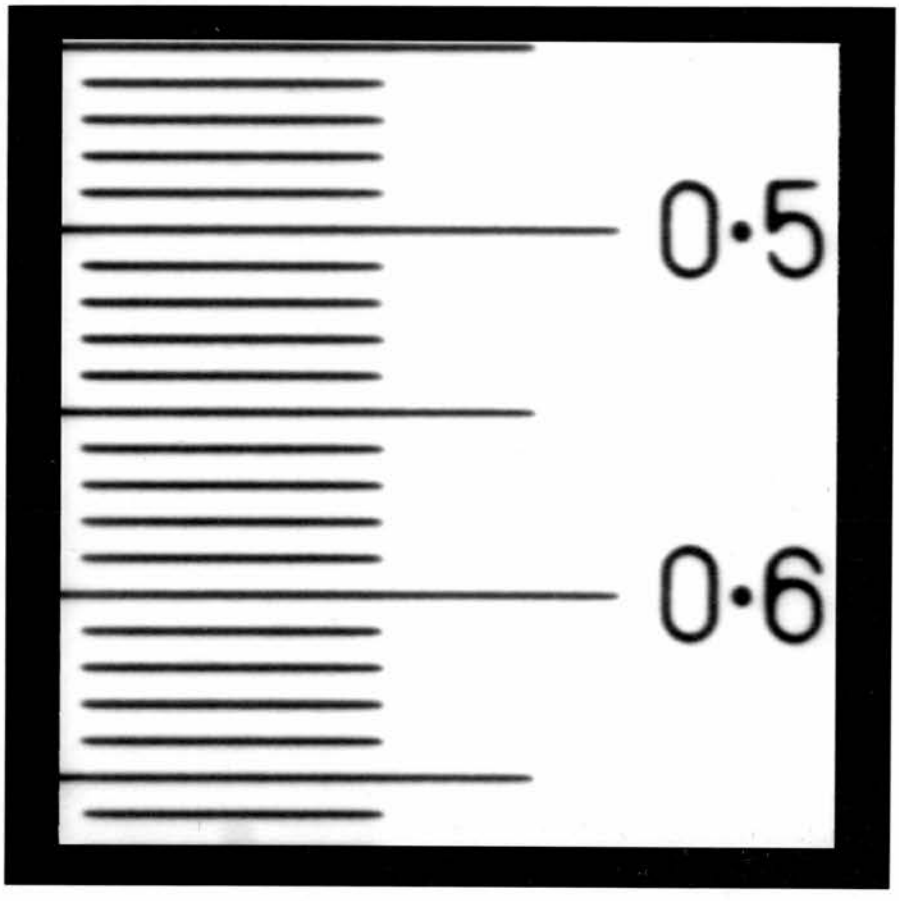


Figure 3.2.3 Micrometer calibration slide sub-divided into 10 μm divisions.

Furthermore, the ease of use of this computerised system was such that its use and its application could be easily mastered, enabling the observer to take large numbers of measurements faster and with greater accuracy, in an accessible and reproducible manner.

In order to audit the reproducibility of the method used, identical fields in few of the slides were re-measured on different days and the values of the different variables recalculated. It could be shown that for the same field and same grey level threshold value, the results were reproducible e.g. if a particular blood vessel or a muscle fibre diameter is measured on different days keeping the grey level threshold value fixed on the grey scale of the machine the results can be reproduced but there was a margin of error which was limited and within acceptable scientific limits for inter- and intra- observer error as shown in section 3.9 of this chapter.

3.4 Measurement of Area and Diameter of Cardiac Muscle Fibres

The H&E stained slides were studied under a x25 objective lens and six randomly selected fields in each slide which appeared to be representative and also free from major artefact, were measured by selecting an appropriate grey level. The grey levels was selected from the darker end of the grey scale (0 to 255) because the muscle fibres are always represented towards the darker end of the grey scale (see figure 3.4.1). In further discriminating the grey levels using threshold values, the grey levels selected were viewed through a green overlay.

The selection of the area occupied by muscle fibres was based on their staining intensity which enabled these areas to be selectively identified in comparison with the areas occupied by blood vessels and by inter muscular connective tissue. Artefactually produced blank spaces within the sections could be positively excluded. If, by chance, parts of blood vessels and areas of connective tissue had taken up the same intensity of staining as the muscle fibres, these could also be specifically excluded from the final tally of measurements.

Figure 3.4.1 Showing selection of cardiac muscle fibres highlighted by manipulation of the grey levels from the left end (darker end) of the grey scale of the IBAS. The muscle fibres appeared green.

By careful scrutiny under the microscope of the area being measured, it was possible to reject selectively other objects which were not to be measured. This could be done by directly identifying with the stylus the interfaces between muscle fibres and the other tissues (see figure 3.4.2 and 3.4.3), with the same colour intensity such as blood vessels, which were not to be measured. This could then be removed from vision and thus availability to the image processing system by pressing an appropriate button on the 'digital tablet'.

The areas that appeared green on the RGB monitor represented the muscle fibres carefully selected from the darker end of the grey scale (see figure 3.4.4). The total area occupied by muscle fibres was calculated automatically by the machine and figures for the total area of the field and the percentage of the area occupied by muscle fibres in that field were also obtained.

The diameter of individual cardiac muscle fibres in slides from each of the regions examined were measured by applying the cursor selectively to each myocyte across the part of the fibre which contained the nucleus (see figure 3.4.5). From the known morphology of cardiac myocytes, the nucleus was considered as a stable landmark, enabling the minimum diameter of the muscle fibre (Fuster et al, 1977; Dick et al, 1982; Fujiwara et al, 1983; Teragaki et al, 1993) to be measured in μm. For each slide between 90 to 180 muscle fibres depending on their size were measured accurately.

3.5 Measurement of the Area occupied by Connective Tissue

For estimating the areas in the slides represented by connective tissue, careful selection of the grey levels was made from the pale end of the grey scale as it is a standard finding in this unit that the connective tissue always tended towards the lighter end of the grey scale. The grey levels selected were viewed through a green overlay. The green overlay applied at the lighter end of the grey scale, and the resulting green areas of connective tissue can be seen on figure 3.5.1.

Figure 3.4.2 Identification of interfaces between muscle fibres and other tissues.

Figure 3.4.3 Muscle fibres separated from adjacent structures by manipulating light pen on the screen to delineate the edges.

Figure 3.4.4 Area of muscle fibres accepted for measurement after direct interactive rejection of any area which was not to be included in measurement.

Figure 3.4.5 Measurement of transverse diameter of cardiac muscle fibres across the transverse plane of the nucleus.

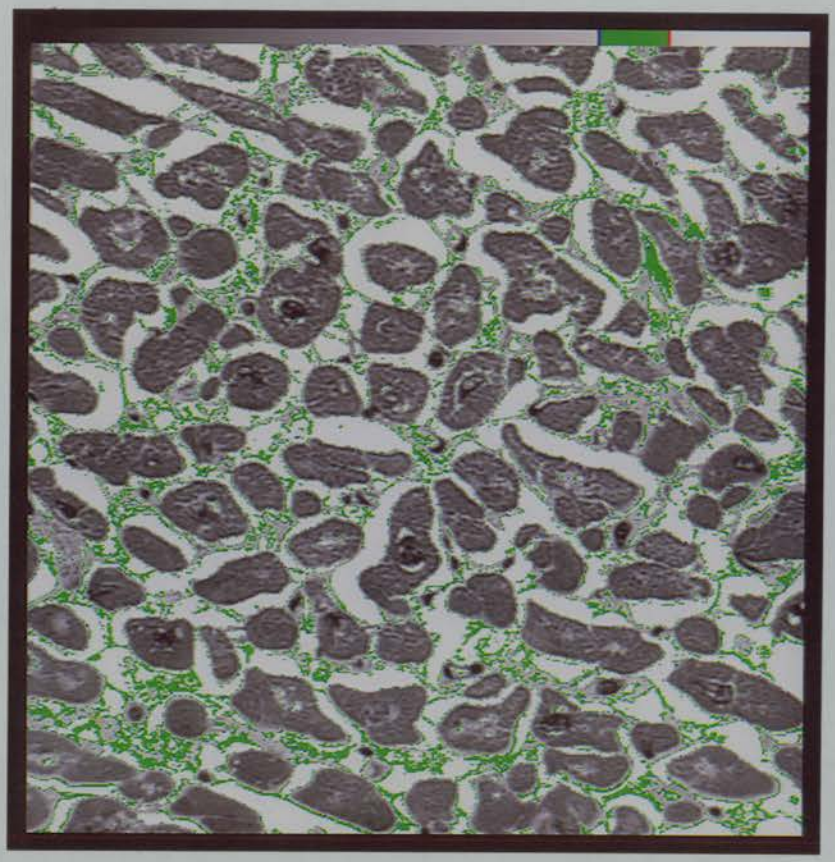


Figure 3.5.1 Selection of connective tissues with grey levels from the right end (pale end) of the grey scale. Connective tissues appear green.

In these areas of connective tissue there were on occasions stained items which could not be included in the connective tissue area assay: these included blood vessels, fibroblasts and muscle fibres that were understained and these are of similar staining intensity to connective tissue. When these objects came up as having the same threshold values as the connective tissue they were excluded from the final measurements. By careful scrutiny under the microscope of the area being measured, one could selectively reject the other objects which were not to be measured. This could be done with the stylus by directly identifying the interfaces between connective tissue and the other objects which were not to be measured. This could then be excluded from the measurement by pressing an appropriate button on the 'digital tablet'.

This, therefore, excluded as much as possible errors which could have resulted from the taking up of stains during slide preparation by areas of no relevance to the study.

The residual and positively accepted area occupied by connective tissue in that field (see figure 3.5.2) was calculated automatically by the machine into total area of connective tissue and the percentage area of connective tissue.

The results for the percentage area of connective tissue, percentage area of muscle and diameters of muscle fibres were obtained separately for each region of the heart examined, from both the alcoholics and the controls.

3.6 Measurement of the Area and Diameter of Cardiac Muscle Fibre Nuclei

As was to be expected, the nucleus showed an uptake of the basic (haematoxylin) dye as compared with the rest of the cardiac muscle fibre. This therefore enabled the nuclei to be specifically chosen by their staining characteristics and by their configuration which enabled accurate measurement of them to be taken.

Figure 3.5.2 An area of connective tissue accepted for measurement.

To estimate the area and diameter occupied by cardiac muscle fibres nuclei, careful selection of the appropriate grey levels was made from the darker end of the grey scale (see figure 3.6.1). The grey levels selected were overlaid with a green overlay. Those areas which appeared green on the RGB monitor represented nuclei. In these areas of nuclei there were on occasions stained items which could not be included in the nuclear measurements: these included blood vessels, fibroblasts, connective tissue and muscle fibres that were deeply stained and of similar staining intensity to nuclei. When these objects came up as having the same threshold values as the nuclei they were excluded from the final measurements. By careful scrutiny under the microscope of the area being measured, one could reject other objects which were not to be measured. Figure 3.6.2 shows the stained nuclei with other objects selectively removed (black regions). The rejected areas were then automatically excluded from the field to be measured by the image processing system, leaving only cardiac muscle fibres nuclei in the field (see figure 3.6.3). Irregular shaped nuclei were also excluded at this stage, the final measurements being performed on the nuclei depicted in figure 3.6.4.

Six randomly selected fields were examined in each slide for each of the cardiac anatomical areas selected for study. The area occupied by nuclei and the minimum diameter of nuclei were calculated in each field automatically by the machine.

The area of nuclei and the minimum diameter of nuclei were calculated separately for each of the six region of the heart examined.

3.7 Measurement of Blood Vessels

To measure blood vessels, slides which had been stained by Van Geison's elastica stain were used. Van Geison's elastica stain depicts such elastic tissue within blood vessels as a black or purplish black wavy line; it also enables the endothelial basement membrane to be identified as a fine continuous line.

Figure 3.6.1 Specific selection of nuclei of cardiac muscle fibres by manipulating the grey levels from the left end of the grey scale. Nuclei appear green.

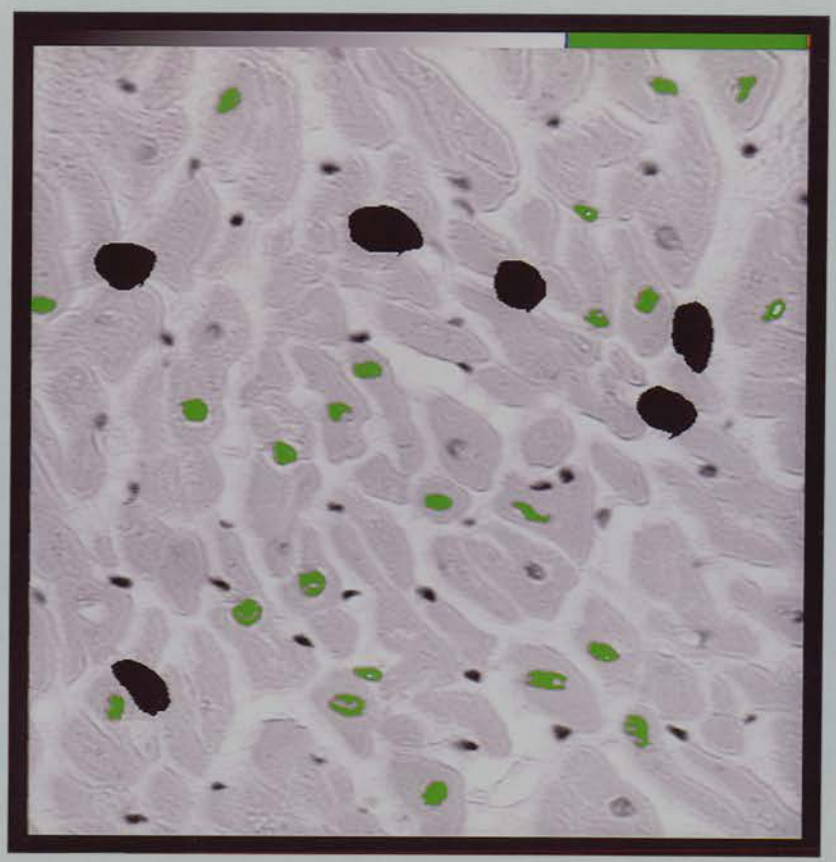


Figure 3.6.2 Rejection of non-nuclear objects by inter-active selection with the light pen.

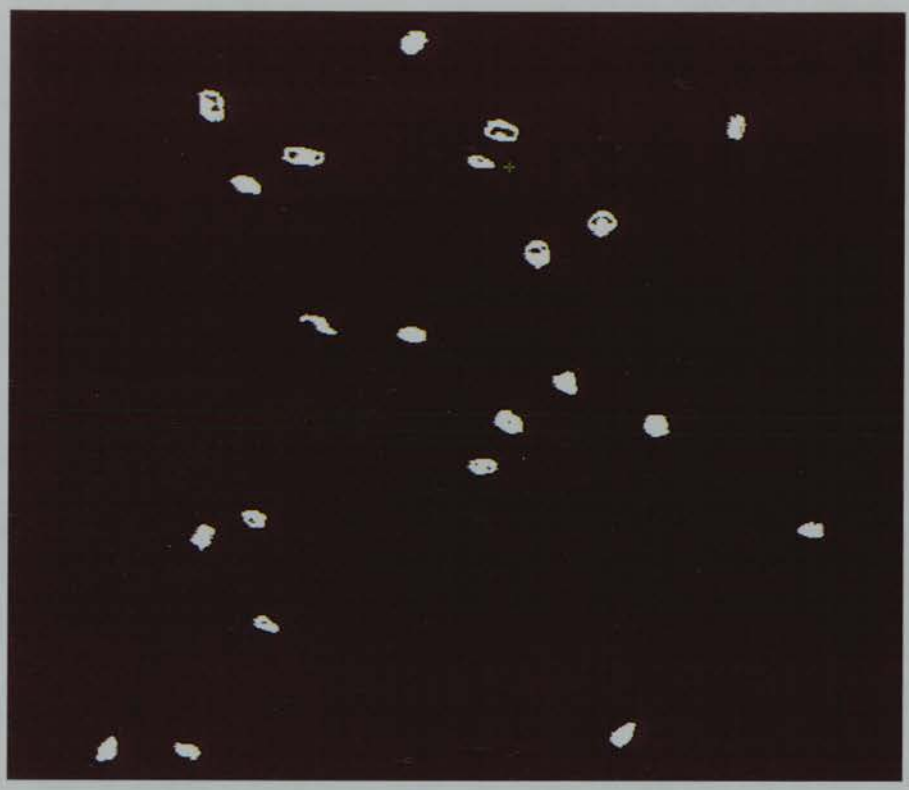


Figure 3.6.3 Identification of objects which did not have an appropriate nuclear configuration.

Figure 3.6.4 A field as finally accepted to contain the nuclei which were to be specifically measured after inter-active rejection of objects that were not to be measured.

The following layers can be identified within the vessel wall (see figure 3.7.1): nearest to the lumen which is variable in size, is a flattened endothelial lining delineated by its basement membrane which in turn is surrounded by a layer of fine connective tissue. A fine layer of concentric aligned elastic tissue separates these two thin layer from the other components of the vessel wall. This portion of the vascular wall is referred to as the INTIMA. This could only be visualised with certainty in muscular arteries, usually of a diameter of 50μm or above; the wavy layer of elastic tissue is known as the internal elastic lamina. The outer boundary of the second coat of the blood vessel i.e. the muscular layer in the wall of an artery is delineated by another concentrically aligned wavy elastic layer, the external elastic lamina, visualised as a thin wavy circumferential line. The thickness of the vessel wall between the internal and the external elastic laminae is the MEDIA. The rest of the vessel wall beyond the external elastic lamina is the ADVENTITIA.

The specific measurements which were taken were limited to arterial vessels and only to those which were sectioned in a roughly transverse direction. Vessels which exhibited tangential or longitudinal cuts were excluded. Therefore, the vessels which were measured were largely of a rounded or oval configuration. For those vessels which were not sectioned in the transverse plane, a mathematical adjustment was made for this artefact. The correction which was introduced into estimates of blood vessels sectioned in this manner, measurements obtained on vessels which were not cut perfectly transversely (as a consequence of the sectioning method), were converted into measurements for transversely cut vessels. The diameter of the hypothetical circle with circumference equivalent to the ellipse was also calculated using the formula:

$$(\text{equivalent diameter})^2 = [(\text{min. diameter})^2 + (\text{max. diameter})^2] / 2$$

The shape of the blood vessels were examined by calculating the eccentricity of the hypothetical ellipses by expressing the minimum diameter as a percentage of the maximum diameter. The formula used is:

$$\text{Eccentricity} = 100 \times (\text{minimum diameter}/\text{maximum diameter})$$

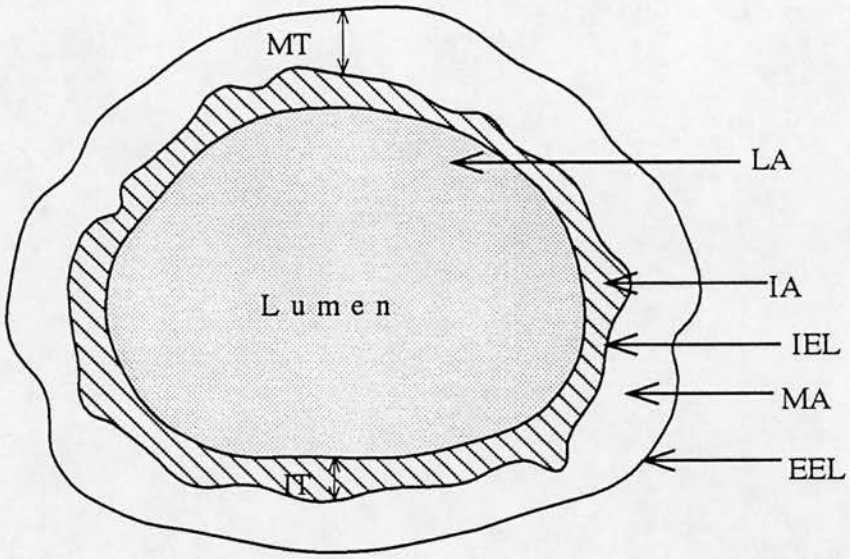


Figure 3.7.1 Diagrammatic representation of different parts of blood vessel. LA= luminal area; IA= Intimal area; IEL= Internal Elastic Lamina; MA= Medial area; EEL= External Elastic Lamina; MT= Thickness of Medial layer; IT= Intimal Thickness.

This gives 100% for circular vessels and smaller values for very elongated shape, and measures the degree of collapse.

The slides were viewed under the x25 objective lens and the images were digitised into 512 x 512 pixels on the IBAS machine using the grey scale ranging from 0 to 255 (black to white). Analysis and processing of the digitised images were carried out by a sequence of macro routines specifically tailored to assess the variables as follows:

The vessels were selected randomly by scanning the slide and ten vessels per slide were measured which had at least two thirds of their IEL and/or EEL intact and easily identifiable. For each specimen the following vascular measurements were carried out (see figure 3.7.2):

3.7.1 Measurements Related to Internal Elastic Lamina (IEL) of Blood Vessels

In these randomly selected blood vessels, by careful manipulation of the cursor and adjustment of the colour scale, it was possible to selectively identify the internal elastic lamina. The internal elastic lamina always produced a higher level of colour intensity and as such could be specifically selected out (see figure 3.7.3). The external elastic lamina could also be visualised although its colour intensity was usually lower than that of internal elastic lamina.

The morphometric system enabled a direct measurement of the total circumferential length of the IEL. In taking such measurement it was possible to exclude positively, by direct interaction, the areas occupied by tissues which although showing up as having similar colour intensity threshold values were not to be measured in this instance by encircling the IEL with the light pen (see figure 3.7.4) and rejecting unwanted objects and other components of vessel wall which automatically disappear from the field (see figure 3.7.5) and hence from the measurement.

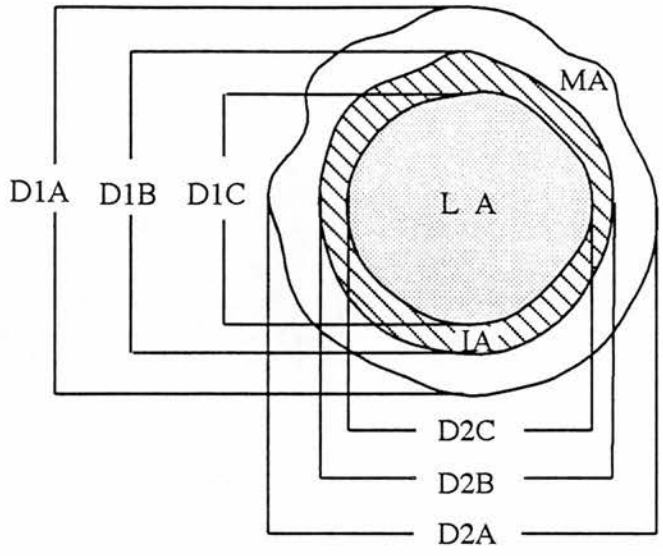


Figure 3.7.2 Diagrammatic representation of measurements made using blood vessel program. D1A & D2A indicate maximum and minimum external diameters of blood vessels; D1B & D2B indicate maximum and minimum internal diameters of blood vessels; D1C & D2C indicate maximum and minimum luminal diameters of blood vessels.

Intimal area= area encircled by IEL- area of the Lumen
Medial area= area encircled by EEL - area encircled by IEL
D max. intima= (D max. IEL- D max. Lumen) / 2
D min. intima= (D min. IEL- D min. Lumen) / 2
D max. media= (D max. EEL- D max. IEL) / 2
D min. media= (D min. EEL- D min. IEL) / 2

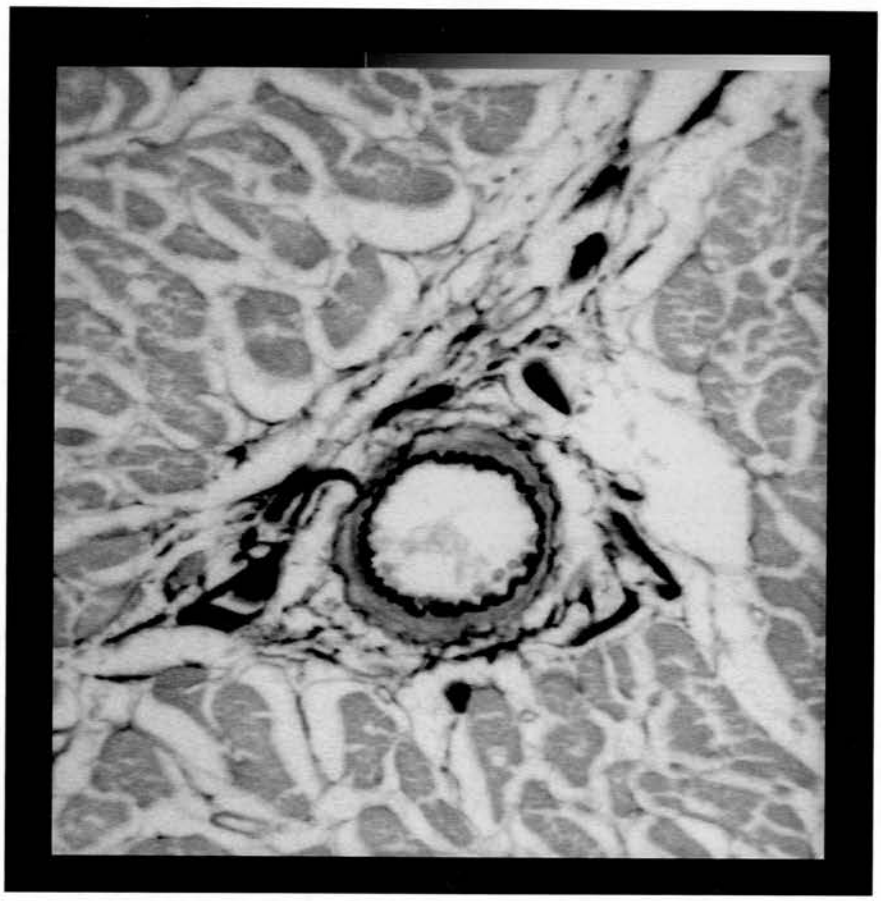


Figure 3.7.3 Selection of the internal elastic lamina (IEL) of a blood vessel within the myocardium by manipulation of the grey levels.

Figure 3.7.4 Exclusion of objects which were not to be included in measurement by drawing around the IEL with the light pen.

Figure 3.7.5 A field finally accepted for measurement with exclusion of all objects not to be included in the measurement.

The total circumferential length of IEL has been considered as a standard variable in relation to other variables which can be used to study the size of the blood vessels. The internal diameter of a blood vessel is considered to be represented by the IEL, as the IEL is not affected by postmortem fixation and slide preparation and its total length remains unaltered even when the vessel collapses or constricts (Fernie and Lamb 1985). The use of this measurement was carefully validated in the study of medial hypertrophy in pulmonary arteries (Fernie and Lamb 1985). Other investigators also have defined artery size in terms of the length of the internal elastic lamina (Cook and Yates, 1972; Yamaki and Tezuka, 1976; Yamaki and Wagenvoort, 1981), however this investigation has used a more accurate technique whereby the measurements were made using highlighted colours. This technique is considered to have advantages over those other techniques. The maximum and minimum diameters of IEL enclosed space and the area of the vessel encircled by IEL were calculated automatically by the machine (see figure 3.7.6).

3.7.2 Measurements Related to Lumen of Blood Vessels

The lumen of blood vessels was measured by manipulating the cursor to choose the appropriate grey levels on the grey scale. In further discriminating the grey levels using threshold values, the grey levels selected were overlaid with a green overlay (see figure 3.7.7). The total area occupied by the lumen could be measured, together with its maximum and minimum diameters which were calculated automatically by the machine (see figure 3.7.8).

3.7.3 Measurement of the Area occupied by the Intima and its Thickness

Having obtained the area and maximum and minimum diameters of the lumen, the area encircled by internal elastic lamina and the maximum and minimum diameters of the area bounded by the internal elastic lamina, the maximum and minimum width of the intima and total area occupied by the intima of the blood vessel were calculated automatically by the machine by subtracting the luminal area

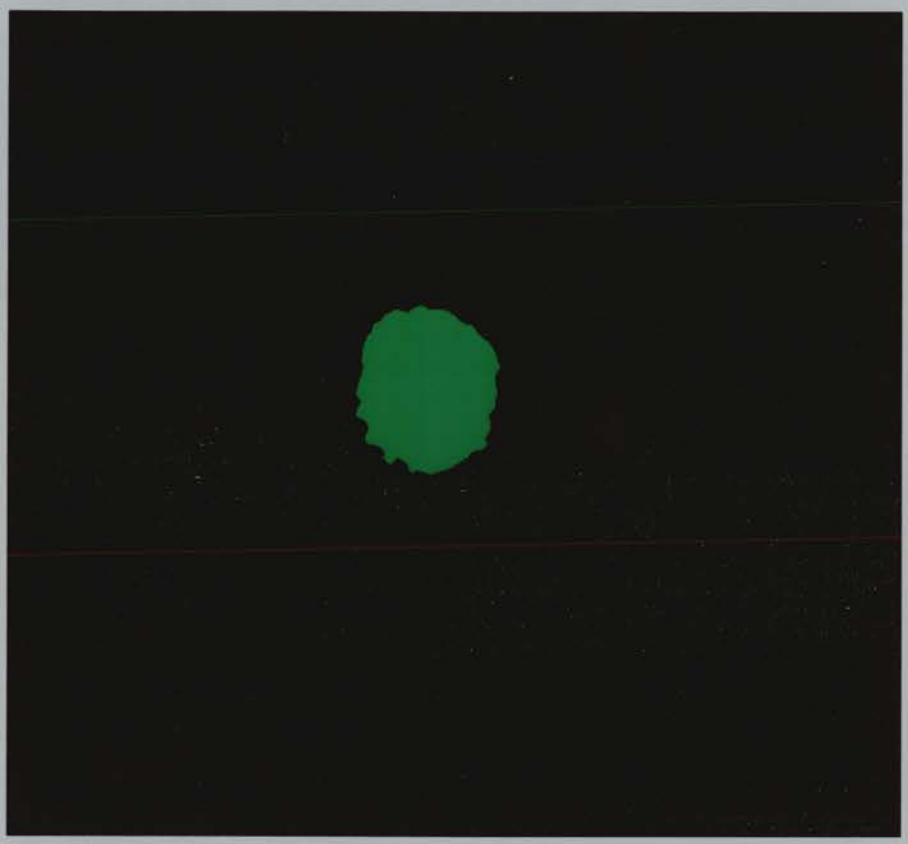


Figure 3.7.6 Measurement of area bounded by IEL and diameters of the vessel wall at the level of the IEL.

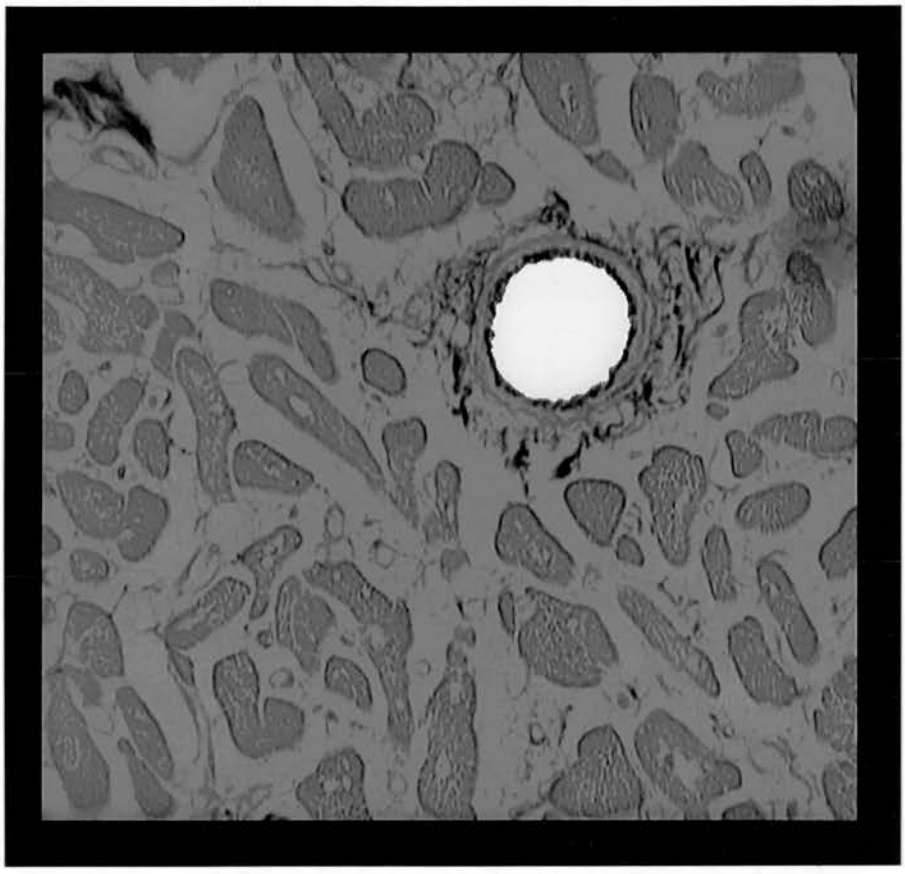


Figure 3.7.7 Selection of lumen of the blood vessel by manipulating grey levels.

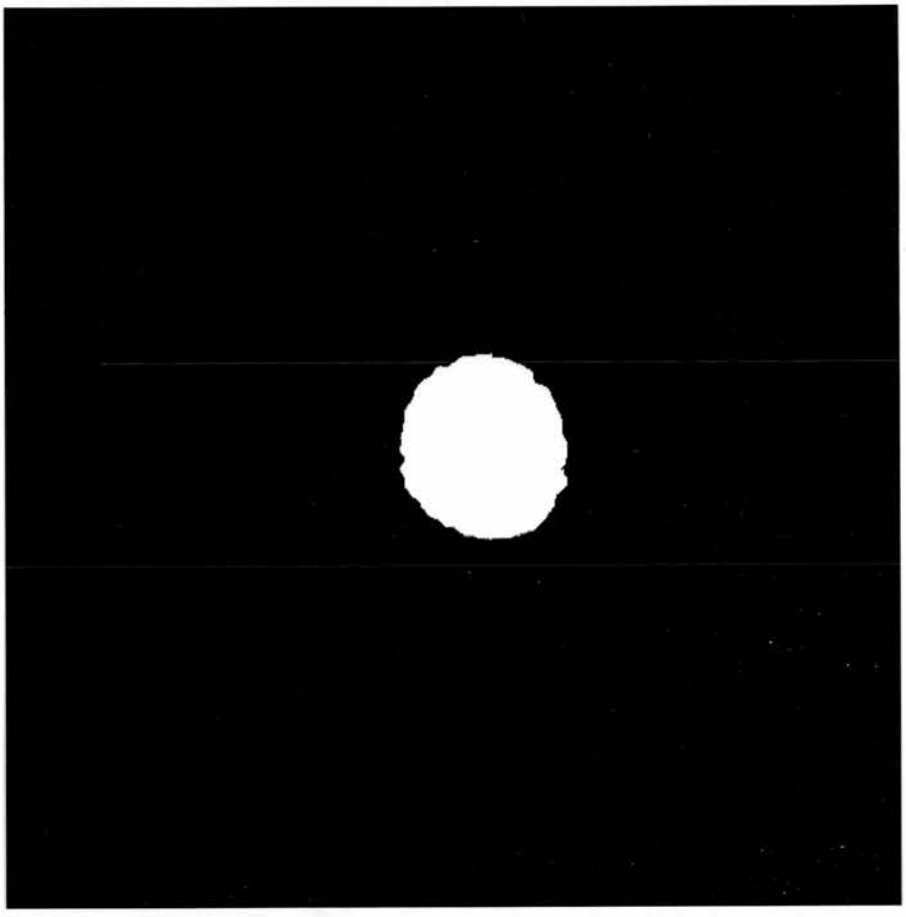


Figure 3.7.8 Final appearance of a field in which measurements of area and diameters of lumen were made.

from the area covered by the internal elastic lamina and the luminal diameters from the diameters of the IEL (see figure 3.7.2).

The intimal measurements were calculated as follows:

Area Intima= (area IEL) - (area Lumen)

D max. Intima= (D max. IEL - D max. Lumen) / 2

D min. Intima= (Dmin. IEL - D min. Lumen) / 2

In the smaller vessels it was not possible to distinguish the intima in that the endothelial cells were thin (1μm to 2μm) and continuous with the internal elastic lamina, so whenever the internal elastic lamina was selected with the grey levels on the grey scale it was not possible to discriminate exactly where the endothelial cells ended and the internal elastic lamina started and to exclude the endothelial cells from the grey levels selection. Therefore, it was decided at the time of measurement to include the internal elastic lamina with the measurement of intima. The area and thickness of the internal elastic lamina was, therefore, always included in the measurement of the area and thickness of the intima.

3.7.4 Measurements related to External Elastic Lamina (EEL) of Blood Vessels

The external diameter of blood vessel is considered to be represented by the external elastic lamina. In this study this was carefully traced by the light pen (see figure 3.7.9) and its circumferential length was measured automatically by the machine. This allowed an automatic calculation by the machine of its maximum and minimum diameters and the area of the vessel wall encircled by EEL.

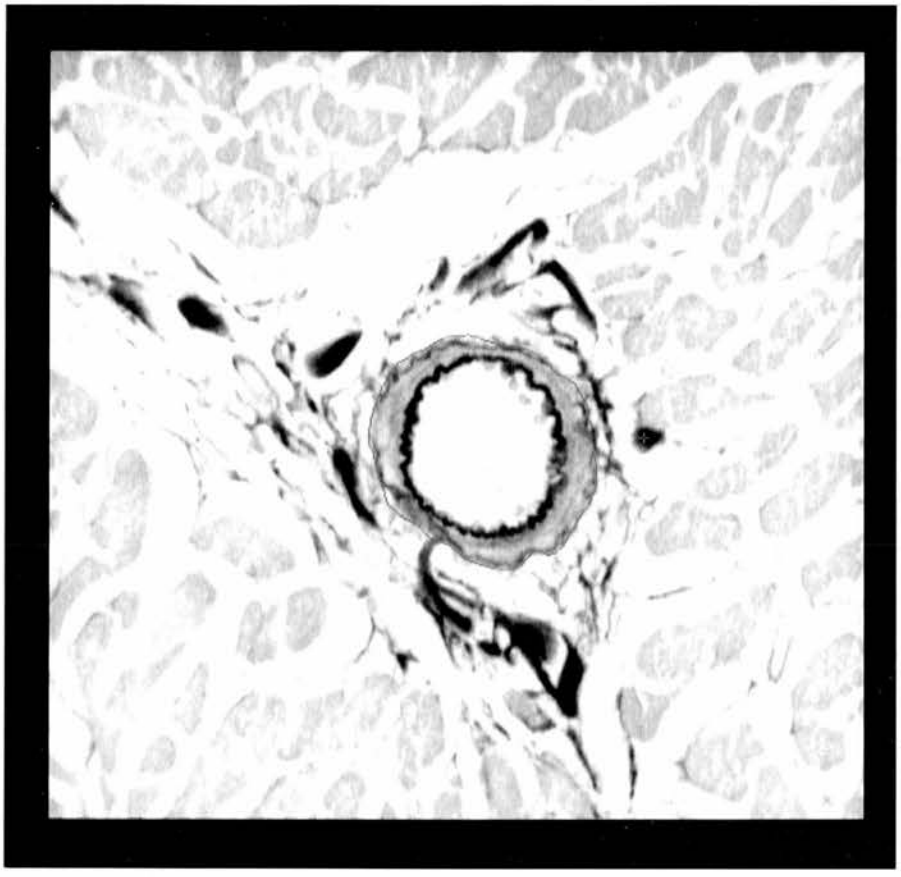


Figure 3.7.9 Tracing of EEL by manipulating the light pen.

3.7.5 Measurement of the Thickness and Area occupied by the Media of Blood Vessels

By subtracting the diameter of the vessel at the level of the internal elastic lamina from the diameter obtained at the level of the external elastic lamina, the maximum and minimum width of the media could also be obtained by the machine. The total area of the media was calculated automatically by the machine by subtracting the area encircled by the internal elastic lamina (IEL) from the area encircled by external elastic lamina (EEL) [see figure 3.7.2]. The medial measurements were calculated as follows:

Area Media= (area EEL) - (area IEL)

D max. Media= (D max. EEL - D max. IEL) / 2

D min. Media= (D min. EEL - D min. IEL) / 2

3.8 Light Microscopy

For the light microscopical study, the H&E stained slides were scanned at several levels of magnification up to x40. The slides stained with PAS were also separately scanned. The PAS staining enabled the demonstration of any deposition of PAS positive material in the intima.

Other microscopical features which were specifically looked for were:

(a) the presence and degree of inflammatory infiltrate at any site within the cardiac sections.
(b) the degree of thickening of the pericardium and endocardium.
(c) the presence of glycogen within myocytes, any depletion thereof or the formation of intracellular clear vacuoles.
(d) the presence and degree of accumulation of lipofuscin (i.e. lysosomal 'wear and tear' brown pigment) which can be identified both in H&E but more so in PAS preparations.

3.9 Reproducibility of Measurements

To assess the reproducibility of the machine measurements, repeat measurements of the same field were carried out on six different days. Reproducibility was measured by the coefficient of variation (CV), defined as the ratio of the standard deviation to the mean, multiplied by 100 to give a percentage.

Coefficient of Variation (CV)= (Standard Deviation / Mean) x 100

3.9.1 Myocyte and Connective Tissue Measurements

Table 3.9.1: Coefficient of Variation (CV) of muscle and connective tissue measurements

Measurement	**CV**
Area of muscle fibre	0.2
Area of connective tissue	0.4
Diameter of muscle fibre	0.2

The coefficient of variations (CVs) for the measurements obtained for diameter and areas occupied by cardiac muscle fibres and areas occupied by connective tissue are acceptable indicating good reproducibility suggesting that measurement error is not an important issue (see table 3.9.1).

As there had to be a marked selectivity in each measurement in the area and diameter of nuclei which were measured on the computer system and these selections were carried out at random in different sittings, a potential for variability was introduced. Even if the sample size were to be increased, it would be difficult to exclude this potential for error unless every single muscle fibre nucleus were to be measured and this is completely impracticable. For this reason an estimation of the coefficient of variation in relation to such measurements would be of little value as it would only reflect the problem of selectivity and bias already alluded to.

3.9.2 Blood Vessel Measurements

Table 3.9.2: Coefficient of Variation (CV) of blood vessel measurements

Measurement	CV
Area of lumen	0.7
Area of intima	1.6
Area of media	1.7
Length of IEL	0.8
Diameter (max) of lumen	0.3
Diameter (min) of lumen	0.4
Diameter (max) of IEL	0.4
Diameter (min) of IEL	0.3
Diameter (max) of EEL	1.1
Diameter (min) of EEL	0.7
Diameter (max) of intima	1.8
Diameter (min) of intima	1.7
Diameter (max) of media	1.5
Diameter (min) of media	1.6

The coefficient of variations (CVs) for the blood vessel measurements are acceptable, indicating good reproducibility, although those for the intima and media are less good than the others (see table 3.9.2). They are all an order of magnitude lower than the CVs found between different fields, suggesting that measurement error is not an important issue in the results for the blood vessels which were obtained on the computer-assisted morphometric system. The only high CV is that for the maximum diameter of the intima. This directly relates to the extremely crinkled IEL encountered at the time of highlighting the internal elastic lamina (IEL) specially in a number of the smaller blood vessels.

3.10 Statistical Analysis

Introduction

In view of the extensive number of measurements obtained, it was essential that their analysis would be carried out appropriately and correlations made looked out from the most suitable statistical point of view. To achieve this several consultations were attended with Dr. Robert A Elton, Medical Statistics Unit of the University of Edinburgh, who has had extensive experience and expertise in statistical methods and analysis applied to biological data. Initially a small data base was constructed into which the measurements obtained were transcribed.

Statistical methods used were chi-squared tests, t-tests, multiple regression, two-factor analysis of variance and F-tests for equal variance.

3.10.1 Analysis of Demographic Data

Demographic characteristics of the two groups were compared by chi-squared, t and F tests.

3.10.2 Analysis of Total Heart Weight

To allow comparison of the data from this study with the investigations of Kitzman et al (1988) who quoted regression equations and residual standard deviations (sd) for the relationship between fixed heart weight and body weight for each sex separately, using logarithmic scales for both variables and approximately normal distribution was suggested; the fixed heart weights and body weights recorded in the study were therefore converted to the logarithmic scale, and **"standardised heart weights"** were calculated from the formula:

(Observed log heart weight - Predicted log heart weight) / residual sd

This measures the number of standard deviations above or below the predicted value, and should have a mean of approximately zero in a sample from a comparable population to that measured by Kitzman et al, with about 95% of values lying between -2 and +2.

Multiple regression was used to compare heart weight measurements between the two study group after adjusting for other factors.

3.10.3 Analysis of Fat-Free Left and Right Ventricular Weights

These weights were compared to the predictions quoted by Hangartner et al (1985) for ventricular weights in relation to total heart weight. Standardised left and right ventricular weights (without fat) were calculated in a similar way to the heart weights above, except that in this case the equations relate the two variables on a non logarithmic scale i.e. on the original scale rather than the logarithmic one.

(Observed LV weight - Predicted LV weight) / residual sd

(Observed RV weight - Predicted RV weight) / residual sd

3.10.4 Analysis of fat content of ventricles

These were calculated by subtraction of the fat-free ventricular weights from the total ventricular weights. The internal comparisons between the two groups (alcoholics and controls) were done using multiple regression.

3.10.5 Fixed versus Fresh Heart Weights

This was done by two sample t-test statistical analysis and the internal comparisons were carried out by multiple regression.

3.10.6 Analysis of Muscle, Connective Tissue and Nucleus Measurements

In addition to the five direct measurements, three further variables were derived for the purpose of analysis.

Percentage connective tissue: the figures for areas of muscle and connective tissue were converted to "percent connective tissue" by dividing the mean area of connective tissue for each field by the sum of the mean areas of muscle and connective tissue and multiplying by 100.

The formula which was employed to calculate the percentage connective tissue is:

Percentage connective tissue = (mean area of connective tissue) / (mean area of muscle + mean area of connective tissue) x 100

This was done to enable a comparison to be made in terms of the space occupied by fibroconnective tissue between the alcoholic hearts and the controls.

Percentage "empty space": this was calculated by subtracting the sum of muscle and connective tissue areas from 100.

Percentage empty space= [100 - (% muscle area + % connective tissue area)]

This will enable an estimate of artefactual spaces within the slides examined.

Relative area of nucleus: this was calculated by taking the ratio of the diameter of the nucleus to that of the muscle fibre, squaring it and multiplying by 100 to give an estimated percentage of muscle area occupied by the nucleus.

Relative area of nucleus= 100x (Diameter of nucleus / Diameter of muscle fibre)^2

Since these formulae were based on the mean figures for each field, it was not possible to calculate a CV for the variation in the derived variables between individual results from each field.

The two study groups were compared by two-sample t-tests, and the comparison of the regions was by two-factor analysis of variance. To compare the four regions of the left ventricle in alcoholics only, a step wise procedure was used, in which regions C, D and F were first compared to one another and then region E was compared to the average of the other three. Region B was also compared to the average of regions C, D, E and F.

3.10.7 Analysis of Blood Vessel Measurements

The data for analysis comprised mean and standard deviation (sd) of ten measurements from each of the five regions of the heart (data for the atrium were omitted from these analyses).

For each measurement except length of IEL, and for each of the five regions, the mean values for alcoholics and controls were compared after adjustment for the mean length of IEL, which was found to vary significantly between the two groups in some cases. This adjustment therefore corrected for any arbitrariness in the choice of blood vessel sizes on different slides, and was achieved by a multiple regression of the measurement means against both length of IEL and group.

Comparison of regions was done by multiple regression of the measurement means against length of IEL and dummy variables representing patient and region effects.

The variability of the ten measurements on each slide was assessed by calculating the coefficients of variation (CV) as the ratio of the standard deviation to the mean (multiplied by 100 to give a percentage), and these were compared between alcoholics and controls by two-sample t-tests.

Chapter 4

Comparison of Heart Weights with Demographic Data

Introduction

Comparisons were made between the observations and measurements taken from the hearts of known chronic abusers of alcohol and control patients. These were matched as much as possible for age and treated in an identical fashion to the study group. The measurements were also carried out in an identical manner.

4.1 Comparison of Demographic Data in Alcoholics and Controls

The comparisons of demographic data in alcoholics and controls are shown in the table 4.1.1.

Table 4.1.1: Mean (sd) demographic details of alcoholics and controls

Variables	Alcoholics	Controls	Significance
Age in years			
(men)	50.5 (11.6)	46.4 (21.1)	NS
(women)	47.8 (11.0)	37.5 (17.3)	NS
Height in cm			
(men)	172.1 (8.1)	174.2 (7.3)	NS
(women)	158.9 (7.5)	166.5 (6.6)	NS
Weight in kg			
(men)	70.3 (16.3)	71.8 (15.5)	NS
(women)	57.7 (10.7)	71.7 (23.4)	NS

4.1.1 Comparison of Age

Table 4.1.1 shows that there was no statistically significant difference between the ages of the chronic alcoholics and of the controls, in both sexes.

In the chronic alcoholism cases, the mean age for males was 50.5 (11.6) years, and the mean age for females was 47.8 (11.0) years. In the control group, the mean age for males was 46.4 (21.1) years and the mean age for females was 37.5 (17.3) years.

Although there was no significant difference in the mean ages, the controls had significantly greater standard deviations in their ages ($F=3.32$, 39 and 27 df, $P<0.001$), reflecting their more variable ages (see table 2.1.1). This relative matching of ages assisted in removing any bias which would have resulted from diseases which tend to affect the heart more frequently with increasing age, in particular atheromatous degeneration of the coronary arteries and the effects thereof on the heart (see table 4.1.1).

4.1.2 Comparison of Gender

Regarding gender matching, in the chronic alcoholism group, the cases were well balanced by gender in that 22 (55%) were males and 18 (45%) were females. Of the twenty eight controls, 24 (86%) were males and 4 (14%) were females.

The percentage of males was significantly greater in controls than alcoholics ($P<0.05$). The gender bias in the controls was dictated by the availability of appropriate cases and it was not possible to recruit into the study more female controls.

4.1.3 Comparison of Body Heights

In the chronic alcoholism cases, the mean height for men was 172.1 (8.1) cm and the mean height for women was 158.9 (7.5) cm. In the control cases, the mean height for men was 174.2 (7.3) cm and the mean height for women was 166.5 (6.6) cm [see table 4.1.1].

There was no significant difference in body heights, in both sexes, in both groups (see table 4.1.1).

4.1.4 Comparison of Body Weights

In the chronic alcoholism group, the mean body weight for men was 70.3 (16.3) kg and the mean body weight for women was 57.7 (10.7) kg. In the twenty eight controls, the mean body weight for men was 71.8 (15.5) kg and mean body weight for women was 71.7 (23.4) kg [see table 4.1.1].

There was no significant difference in body weights, in both sexes, in both groups (see table 4.1.1).

4.1.5 Body Mass Index (BMI)

When the 'body mass index' is calculated from the figures as shown in table 2.1.1, given that no statistical significant difference was demonstrable between the two study groups in relation to either body heights or body weights in both sexes (see table 4.1.1), no significant difference is to be expected in this calculated parameter.

4.1.6 Occupation

The distribution of occupation in the chronic alcoholism group and in the non-alcoholic group is shown in table 4.1.2.

Table 4.1.2: Distribution of occupation (with %)

Occupation	**Alcoholics**	**Controls**
Manual	9 (22.5%)	9 (32.1%)
Clerical	3 (7.5%)	2 (7.1%)
Professional	3 (7.5%)	7 (25%)
Unemployed	12 (30%)	1 (3.5%)
Retired	6 (15%)	7 (25%)
Housewife	7 (17.5%)	2 (7.1%)

Of the 40 alcoholics, 30% were unemployed; 22.5%, manual workers; 17.5%, housewives; 15%, retired; 7.5%, clerical staff and 7.5% were professional. In the control group, 32.1% were manual workers; 25%, retired; 25%, professional; 7.1%, clerical staff; 7.1%, housewives; 3.5% were unemployed (see table 4.1.2).

The general impression from these known occupations is that the two groups appear to be generally compatible and comparable.

4.2 Analysis of Heart Weights

4.2.1 Fresh versus Fixed Heart Weight

Fixation in formalin over a prolonged period is known to cause a decrease in the total weight of all solid organs as compared to the fresh weights which were measured at the time of autopsy.

In this series fixation in 10% buffered formalin for variable periods over two weeks produced a diminution (mean 6%) of the total heart weight in the controls and also a decrease (mean 5%) of the total heart weight in the hearts from alcoholics. Fixed heart weights were significantly lower than fresh heart weights in both groups ($P<0.001$).

However, no significant difference was found between the two groups when the weight decrease in fixed heart weight is expressed as a percentage of the fresh heart weight ($t=0.95$). The mean (range) of this percentage was 94 (85-97) in the controls and 95 (81-106) in the alcoholics. This suggests that both the groups being studied shared a shrinkage of their weights but this appeared to be similar in either of the two groups indicating that reliable comparisons can be made between both the fresh and the fixed heart weight in these two groups.

4.2.2 Mean Fresh and Fixed Heart Weights in the Controls and Alcoholics

The mean weights of the fresh and formalin fixed hearts in the controls and alcoholics are shown in the table 4.2.1.

Table 4.2.1: Mean (sd) heart weights (g) in the two groups.

	Alcoholics	**Controls**
Fresh heart weight	368 (99)	348 (54)
Fixed heart weight	350 (94)	328 (54)

As these heart weights do not take into account body weight and body height, and the age of the deceased, no statistical correlations were carried out at this stage.

4.2.3 Comparison of Total Fixed Heart Weight with Published Data

The standards used for comparing the data from this investigation for fixed heart weights were taken from the published figures found in the paper by Kitzman et al (1988). It was not felt necessary to use the childhood standards of Scholz et al (1988) for the few individuals in the present study who were less than 20 years old, since these were all in their late teens and the differences in weights from the 20 year old was minimal.

Mean (sd) standardised heart weights scores in the controls were 0.16 (0.77) for males and 0.22 (0.73) for females, and neither of these was significantly different from zero ($t=0.99$, 23 df and $t=0.61$, 3 df respectively), indicating that the controls in this study were comparable to the Kitzman population and therefore within the normal range. As shown by Kitzman et al (1988) in their population, this study also indicated that **body weight** is a better univariate predictor of normal heart weight than either body height or age. In figure 4.2.1, a correlation that is shown by plotting total fixed heart weight against body weight (see figure 4.2.1).

In the alcoholics, the corresponding mean (sd) standardised heart weights scores were 1.15 (1.15) for males and 0.70 (0.85) for females, giving $t=4.68$, 21 df ($P<0.001$) and $t=3.48$, 17 df ($P<0.01$) respectively. Thus alcoholics showed significantly enlarged hearts relative to body weight in both sexes.

The results are illustrated in Fig. 4.2.2, which shows a plot of standardised heart weight against body weight by group. As shown in the plot, none of the 28 controls have their heart weights above the limit of +2, where as 8 of the 40 alcoholics show their heart weights above the limit of +2 and hence cardiac enlargement according to this limit (see figure 4.2.2).

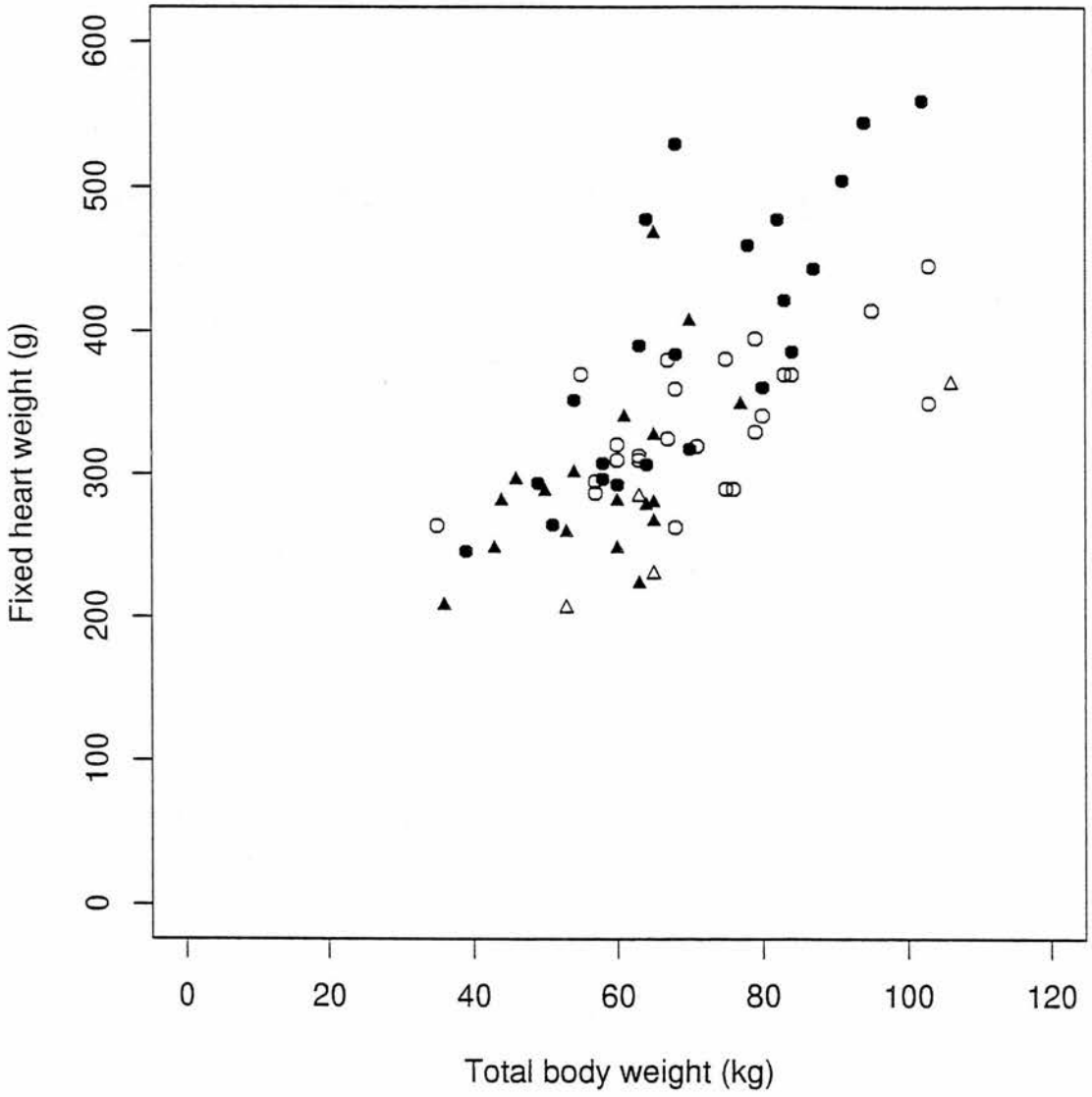


Figure 4.2.1: Plot of fixed heart weight against total body weight. Symbols: ●=alcoholic male; ▲= alcoholic female; O= control male; Δ= contol female; and for subsequent tables.

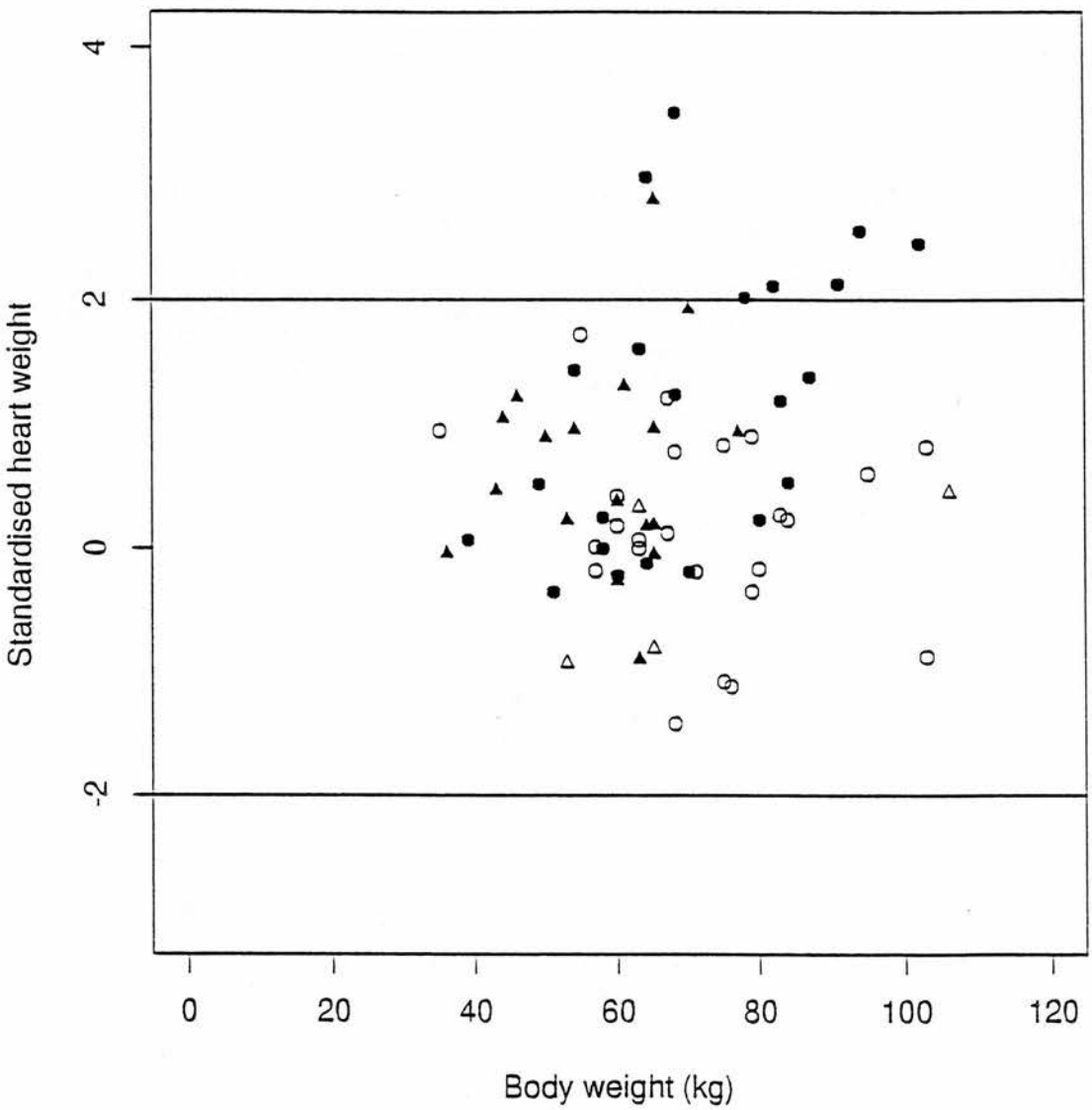


Figure 4.2.2 A plot of "standardised heart weights" against body weight.

When standardised heart weights were plotted against age by group, the figure shows that there was no strong trend to be observed with age changes in the heart weights when adjusted for body weight (Fig 4.2.3).

A direct internal comparison between the controls and alcoholics was also carried out by multiple regression of log heart weight on log body weight and group, and this showed that the alcoholics had significantly increased heart weight adjusted for body weight than controls ($t=2.91$, 65 df, $P<0.01$).

Multiple regression studies also showed that neither height nor body mass index were significantly associated with heart weight after adjustment for the effect of body weight.

4.2.4 Left and Right Ventricular Weights With and Without Fat

The mean left and right ventricular weights with and without fat both in the controls and in the alcoholics are shown in the table 4.2.2.

Table 4.2.2: Mean (sd) non-fat free and fat-free weights (g) of the left and right ventricles in two groups.

	Alcoholics	**Controls**
LV weight with fat	175 (54)	165 (34)
LV weight less fat	153 (47)	147 (29)
RV weight with fat	97 (30)	78 (16)
RV weight less fat	64 (18)	54 (12)

In figure 4.2.4, which shows a good correlation when total left ventricular weights plotted against fixed heart weight (see figure 4.2.4). Similarly, in figure 4.2.5, a good correlation is shown when total right ventricular weight is plotted against fixed heart weight (see figure 4.2.5).

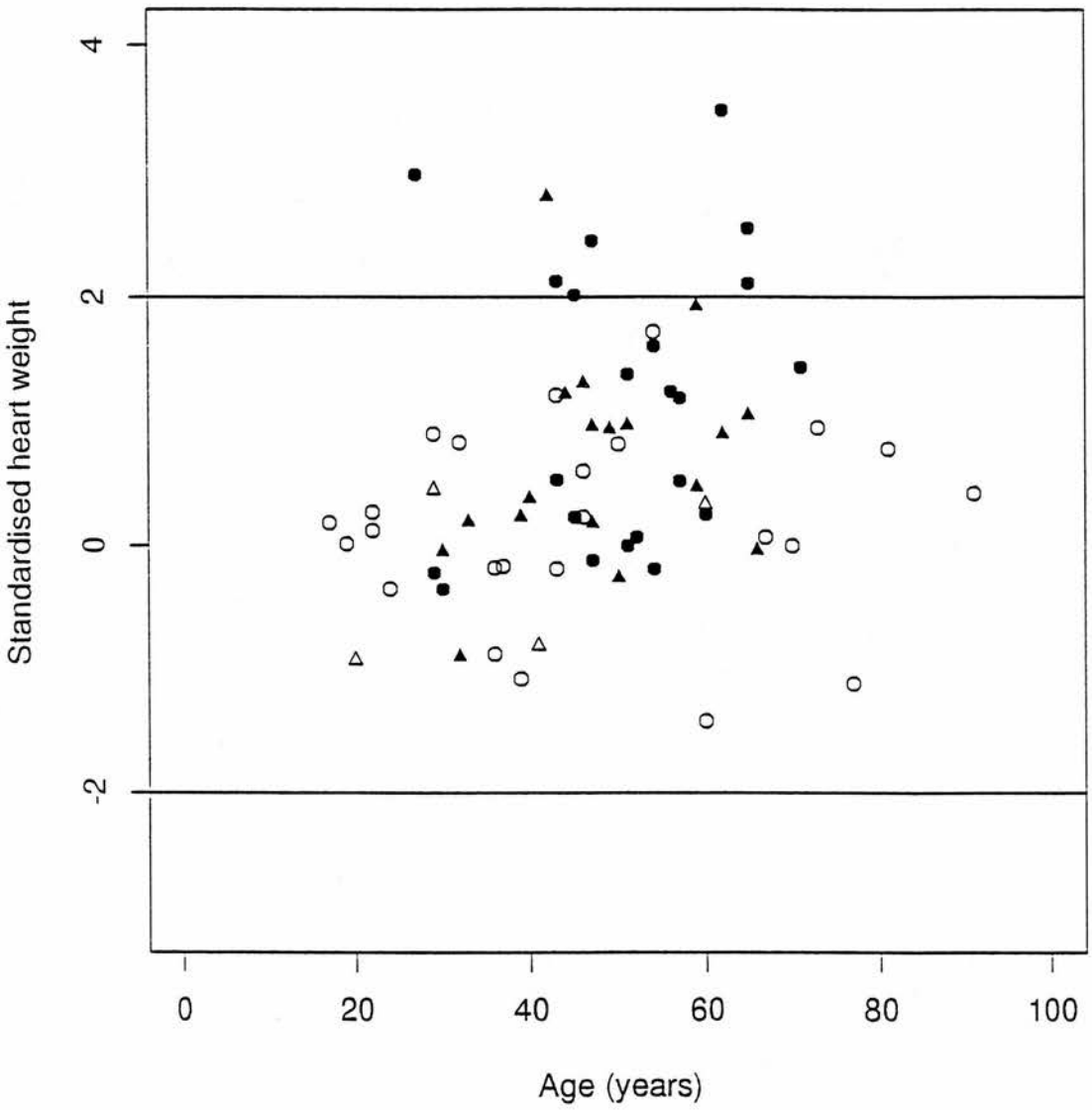


Figure 4.2.3 Plot of "standardised heart weights" against age by group.

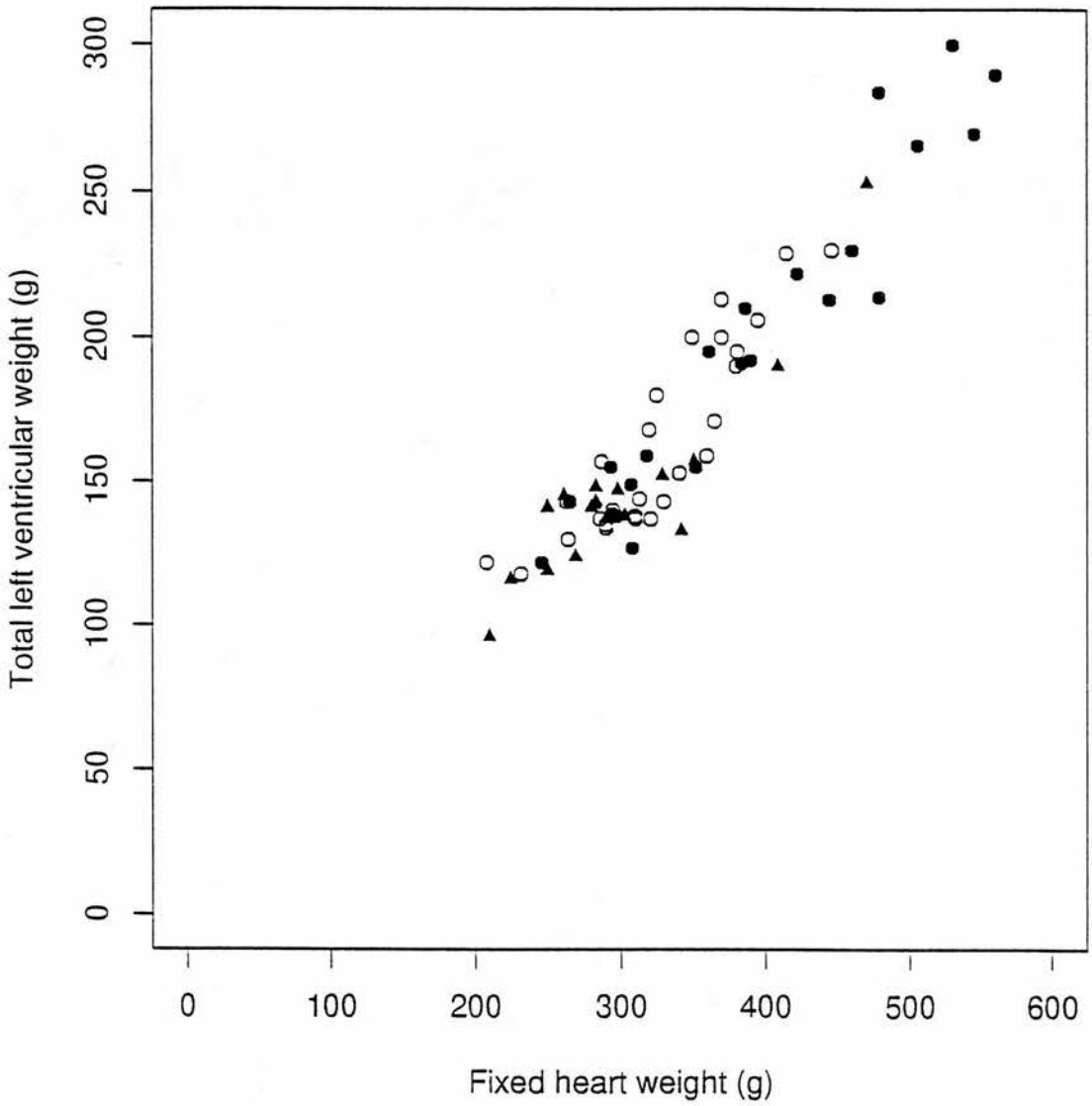


Figure 4.2.4 Total left ventricular weight (with septum) plotted against fixed heart weight.

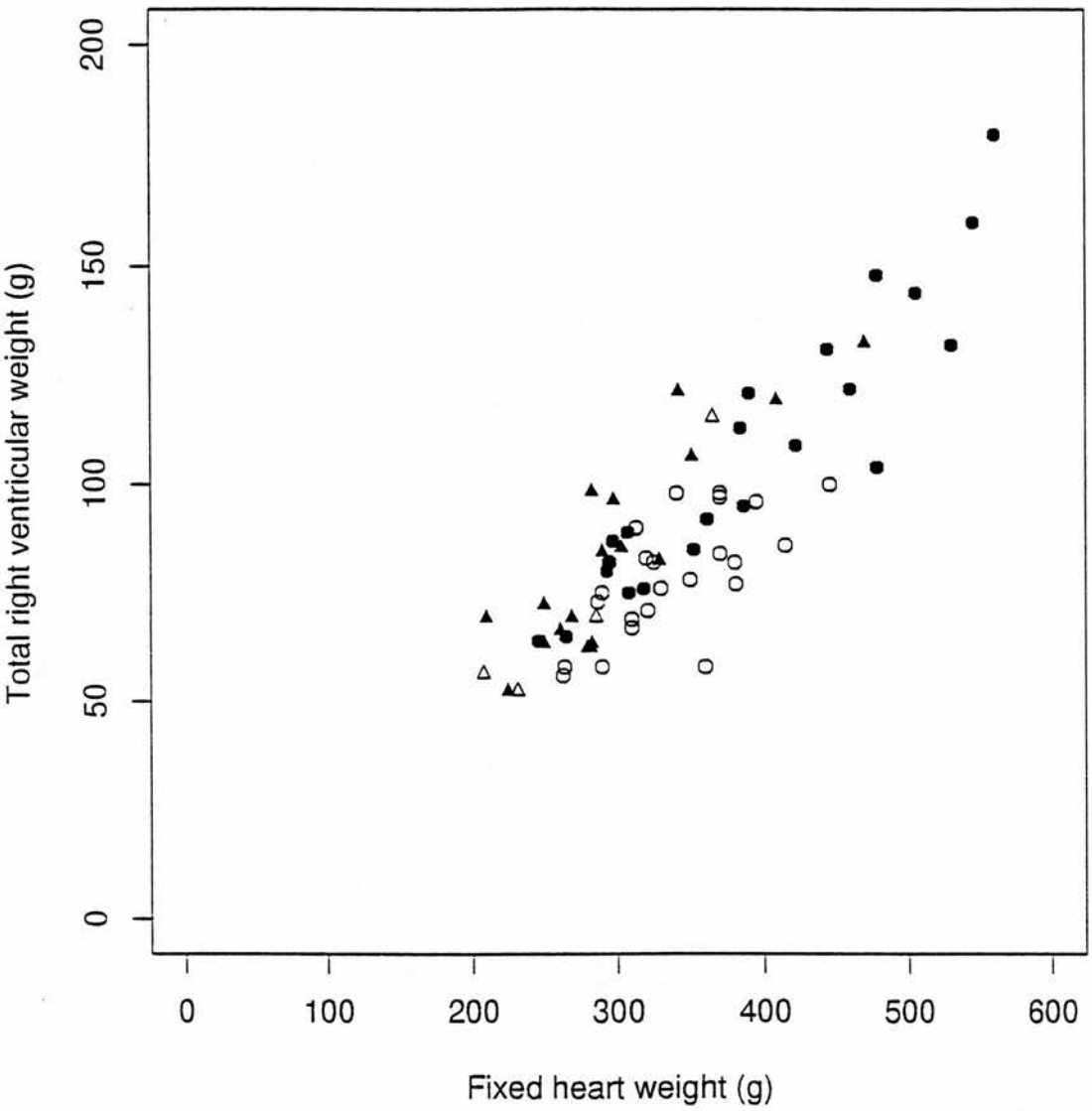


Figure 4.2.5 A plot of total right ventricular weight against fixed heart weight.

As these ventricular weights do not take into account body height, body weight and age of the deceased, no other statistical analysis were carried out at this stage.

4.2.5 Standardised Fat-Free Left and Right Ventricular Weights Compared with Published Data

The mean (sd) standardised left ventricular weights in the controls was 0.22 (0.46) and in the alcoholics was 0.11 (0.48), neither of which was significantly different from zero. In the internal comparison by multiple regression, there was no significant difference between the two groups.

By contrast, the mean (sd) standardised right ventricular weights was 0.41 (0.68) in controls and 0.97 (0.61) in alcoholics were both significantly greater in this study than in the literature predictions ($t=3.19$, 27 df, $P<0.01$ and $t=9.96$, 39df, $P<0.001$ respectively). In the internal comparison, the alcoholics had significantly greater right ventricular weights than the controls after adjustment for total heart weight ($t=2.75$, $P<0.01$).

In view of this last finding, the heart weight remaining after subtraction of the right ventricular weight was calculated, and it was found by multiple regression that the heart weight remaining was significantly greater in alcoholics than in controls after adjusted for body weight ($t=2.76$, $P<0.01$). Thus not all of the excess total heart weight in alcoholics could be explained by the excess right ventricular weight.

Finally, multiple regression studies of left and right ventricular weights on body weight and total fixed heart weight were carried out and the control group showed that body weight did not significantly predict either of the ventricular weights after adjustment for heart weight, which is a better predictor for either of the ventricular weights.

The results are illustrated in figures 4.2.6 and 4.2.7, which shows a good correlation when fat-free left and right ventricular weights were plotted against fixed heart weight (see figures 4.2.6 and 4.2.7).

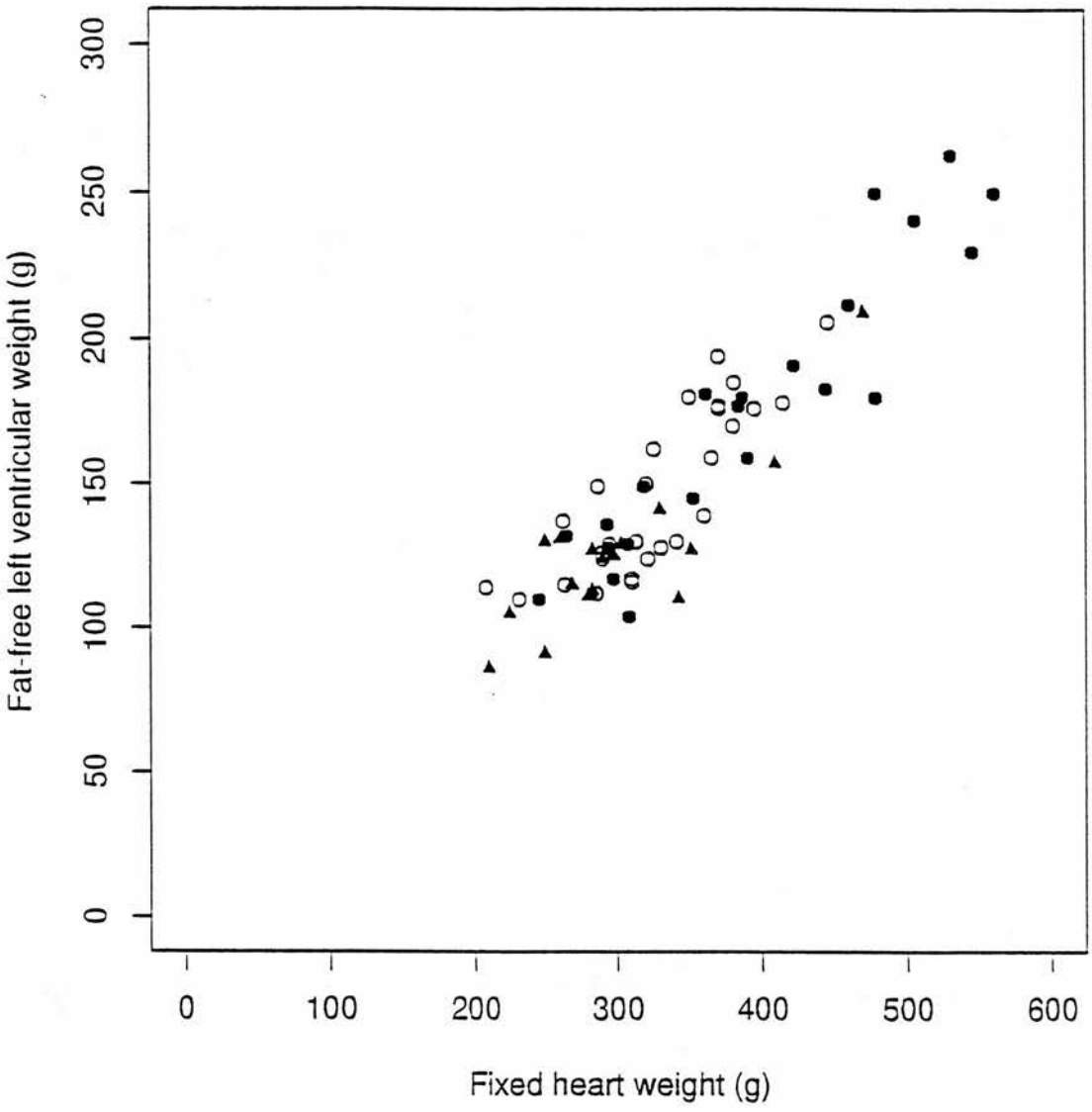


Figure 4.2.6 A plot of fat-free left ventricular weight against fixed heart weight.

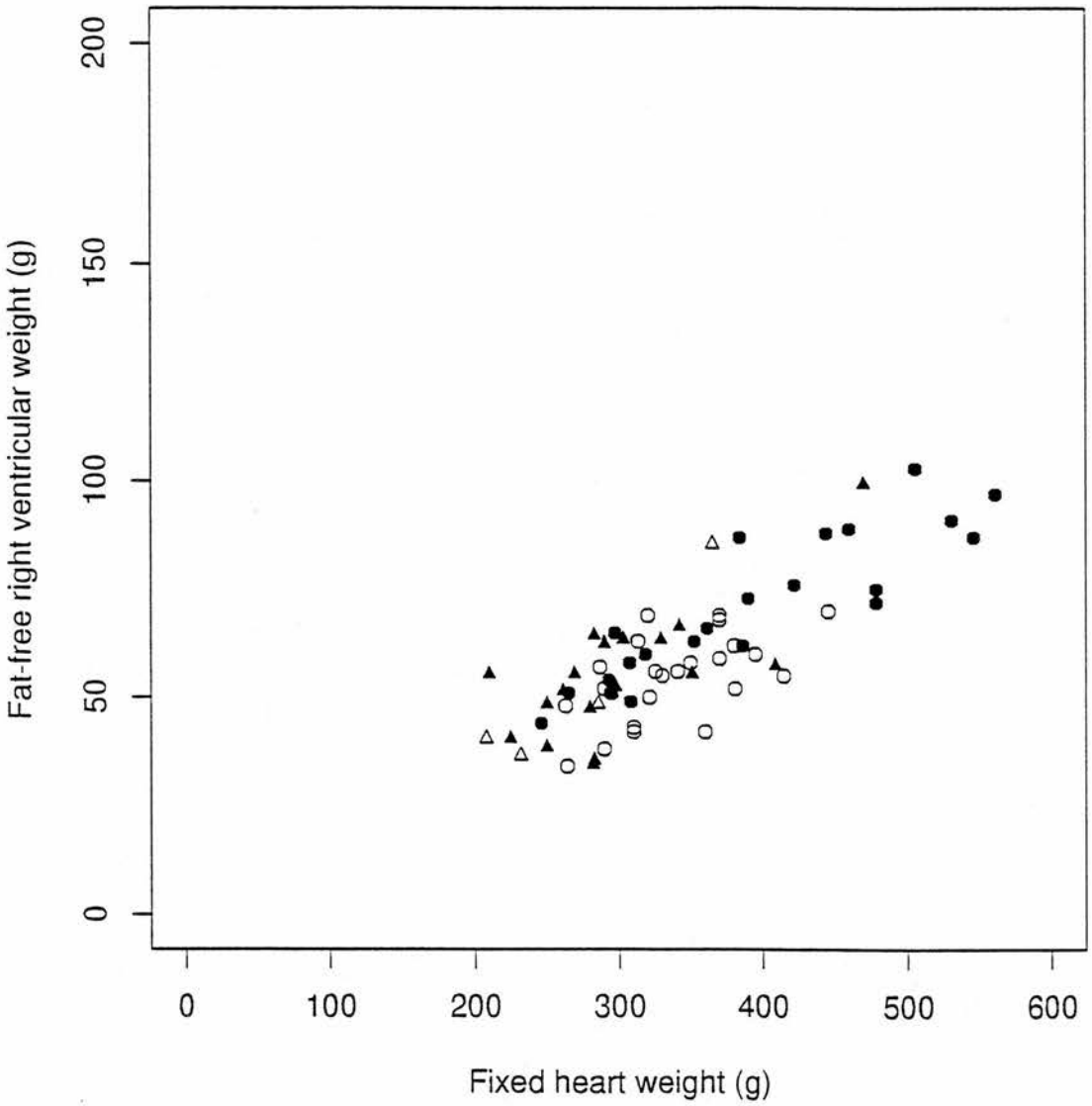


Figure 4.2.7 Fat-free right ventricular weight plotted against fixed heart weight.

All but one of the right ventricular weights in the controls are comparable to the data published by Lamb (1973) and similarly all left ventricular weights are within Lamb's criteria for normal left ventricular weights. All the left ventricular weights are comparable to the published data by Hangartner et al (1985), but the right ventricular weights in this study sample are bigger than those of the Hangartner et al (1985).

4.2.6 Ratio of the Left Ventricular Weight (with septum) to the Right Ventricular Weight

In studying the relationship between the weights of the right and the left side of the heart, the publications by Fulton et al (1952) and Lamb (1973) were used as the bases on which such comparison was made. Fulton et al (1952) indicated in a normal autopsy population that the ratio between the left and the right ventricular weight should be between 2.3:1 and 3.3:1. In this series Fulton et al (1952) departed from previous practice in that the septum was included in the left ventricular weight measurement. Lamb (1973) also followed the new convention and included the septum with the left ventricular measurement and found that the normal ratio was (2:1). The ratio of the left ventricular weight (with septum) to the right ventricular weight in this study are shown in the table 4.2.3.

Table 4.2.3: Mean (sd) ratio of left ventricle to right ventricle.

	Left Ventricle	**:**	**Right Ventricle**
Controls	2.71 (0.46)	:	1
Alcoholics	2.40 (0.40)	:	1

In the alcoholic group, 15 hearts showed a left to right ventricular weight ratio of <2.3 : 1 and in 1 alcoholic heart the left to right ventricular weight ratio was >3.3 : 1. In the control group, 4 hearts showed a left to right ventricular weight ratio of <2.3 : 1 and in 4 control hearts the left to right ventricular ratio was >3.3 : 1 outside Fulton et al (1952) range. In the alcoholic group, 5 hearts showed a left to right ventricular weight ratio of <2 : 1; and in 1 control heart the left to right ventricular ratio was <2 : 1 outside Lamb's (1973) range.

In the alcoholic group, 15 hearts showed a left to right ventricular weight ratio of <2.3:1 which indicates a right ventricular bias in weight likely to be due to hypertrophy of the muscle. In one alcoholic heart the ratio was >3.3:1 which tends to suggest left ventricular hypertrophy. In the control hearts, eight gave results which fall outside the Fulton et al (1952) range, 4 being less than 2.3:1 and 4 other above 3.3:1.

Using Lamb's (1973) criteria the left to right ventricular weight ratio in five alcoholic hearts were less than (2:1) suggesting right ventricular hypertrophy. These all fall within the right ventricular weight biased group in the Fulton et al (1952) comparison. In only one of the controls in which Lamb's ratio was employed could right ventricular hypertrophy be identified.

Figure 4.2.8 shows a correlation between left and right ventricular weights in both groups, and also that right ventricles are enlarged in many alcoholics when right ventricular weights were plotted against left ventricular weights.

4.2.7 Contribution of Ventricular Subpericardial Fat

To estimate the amount of fat which was present subpericardially in the ventricles of hearts, the fat-free ventricular weights were subtracted from the total ventricular weights.

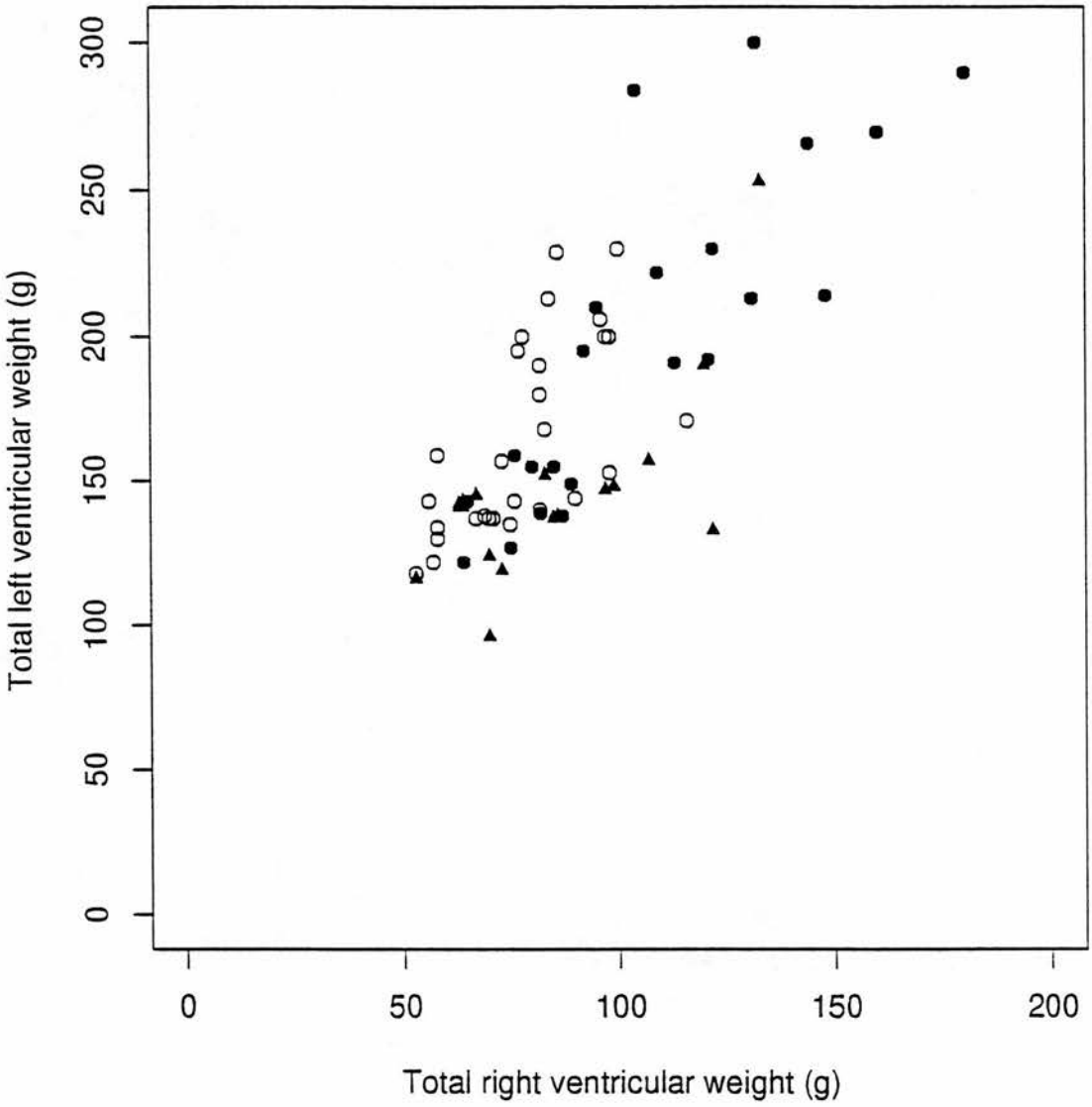


Figure 4.2.8 Total left ventricular weights plotted against total right ventricular weights.

Table 4.2.4: Mean (sd) percentage of fat and non-fat in total fixed heart weight (g) from the Ventricles of the heart. Significance refers to two-sample t-test for group difference.

	Alcoholics	**Controls**	**Significance**
LV fat	6.3 (2.3)	5.3 (2.1)	NS
LV non-fat	43.5 (4.7)	44.9 (5.1)	NS
RV fat	9.1 (3.4)	7.3 (1.9)	$p<0.05$
RV non-fat	18.5 (2.8)	16.6 (2.8)	$p<0.05$

The combined fat weight as a percentage of total heart weight was significantly higher ($P<0.05$) in alcoholics (mean 15%) than in controls (mean 12%).

The mean (sd) right ventricular fat weight was 7.3 (1.9) and the right ventricular non-fat weight was 16.6 (2.8) in the controls and the corresponding right ventricular fat weight was 9.1 (3.4) and right ventricular non- fat weight was 18.5 (2.8) in the alcoholics. Both right ventricular fat and right ventricular non-fat comprise a significantly larger percentage of total heart weight in alcoholics than in controls (see table 4.2.4).

The mean (sd) left ventricular fat weight was 5.3 (2.1) and left ventricular non-fat weight was 44.9 (5.1) in the controls and in the alcoholics the left ventricular fat weight was 6.3 (2.3) and left ventricular non-fat weight was 43.5 (4.7). In the internal comparisons using multiple regression, there was no significant difference between the two groups (see table 4.2.4).

Figure 4.2.9 shows the contribution of epicardial fat in an alcoholic heart (see figure 4.2.9).

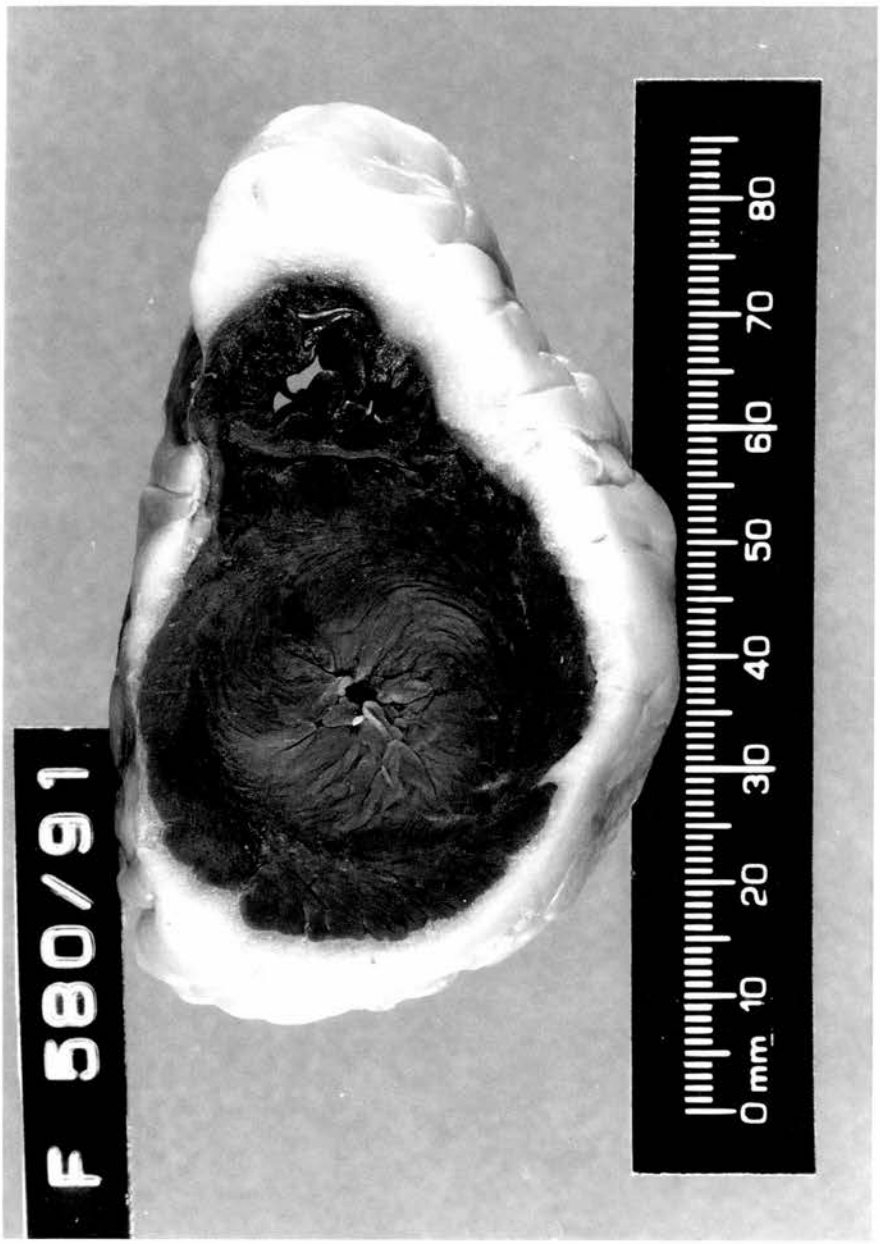


Figure 4.2.9 Transverse section through the heart to demonstrate the contribution of subpericardial fat to the size of an alcoholic heart.

Chapter 5

Other Morphological Abnormalities In Alcoholic Hearts

Introduction

In addition to morphometric studies each of the hearts was examined thoroughly for any pathological abnormalities which could not be measured, both macroscopically and microscopically.

It was obvious on sectioning some of the hearts that the right ventricular chamber in 11 (27%), and the left ventricular chamber in 4 (10%) of the alcoholic hearts were distended and dilated (see figure 5.1) compared with the controls (see figure 5.2), and also the right ventricular dilatation was more conspicuous than the left ventricle (see figure 5.3). Atrial dilatation was present only in 4 (10%) of the cases.

No specific measurements were made of the diameter of atrio-ventricular rings as this is considered by many pathologists to be fraught with problems (Chapman, 1964; Roberts, 1970; Westaby et al, 1984) and the only measurements made were the weights of the ventricular chambers, which gives little indication of the degree of dilatation. The measurement of ventricular wall thickness was not done given the difficulties which also are inherent in obtaining useful and reproducible accurate measurements (Reiner et al, 1959; Lamb, 1973; Dadgar et al, 1979; Hangartner et al, 1985) on which an opinion as to the degree of ventricular wall dilatation can be made.

On microscopical examination, the visceral pericardium showed some excess of fibrous tissue in 5 (12%) of the alcoholic hearts. No active inflammatory changes were observed in this layer but a few aggregates of lymphocytes were present in 6 (15%) of the alcoholic hearts (see figure 5.4). The significance of this finding is doubtful in that one otherwise completely normal heart showed these focal changes as an incidental finding.

The endocardium was somewhat thickened due to the deposition of collagenous fibrous tissue in 11 (27%) of the cases (see figure 5.5) and in a further case this was due to deposition of eosinophilic structureless material which was not amyloid. This may be in keeping with vascular-induced damage to the most ischaemically vulnerable portion of the heart (Eng et al, 1987; Hittinger et al, 1989 and 1990).

In addition to the morphological changes seen in the appearance and size of cardiac myocytes, histological examination confirmed the presence of myocyte hypertrophy (see figure 5.6) in the alcoholics than in the non-alcoholic controls (see figure 5.7) and also an excess of parenchymal connective tissue (see figure 5.8) which appeared to have a perimyocytic distribution in about 5 (12%) of the alcoholic hearts studied (see figure 5.9) compared with the controls (see figure 5.10).

Lipofuscin deposition is a very variable phenomenon but it could be identified with constant regularity in all alcoholic hearts and was extremely conspicuous. Both human and animal studies have shown that an accumulation of lipopigment ('wear and tear' pigment) increases in the heart with cellular ageing and chronic ethanol exposure (Strethler et al, 1959; Sohal and Brunk, 1990; Jaatinen et al, 1993).

No other myocardial degenerative changes such as vacuolisation or deposition of lipids could be seen in the study cases with the exception of one case (see figure 5.11).

Within the interstitial tissue tiny foci of chronic inflammation was observed in one case only and their significance is negligible.

Subpericardial fatty infiltration of the outer layer of the myocardium (see figure 5.12) was present in about half (42%) of the alcoholic hearts examined and indeed this was one of the reason why fat-trimmed heart weights were used in the comparisons between the two groups in this study. This is again a very variable phenomenon which can be seen at autopsy in association with no specific conditions (Lamb, 1973; Womack, 1983; Hangartner et al, 1985)

In the hearts which on morphometric studies measurable arterial and arteriolar changes were found, it was not always possible to identify similar changes

under the microscope in all the cases indeed. Excess perivascular connective tissue (see figure 5.13) was observed in 10 (25%) of the alcoholic hearts examined and thickening of the medial layer was observed in some of the arteriolar vessels (see figure 5.14) in 5 (12%) of the alcoholic cases. Subendothelial "humps" (see figure 5.15) were also present in some of the arterioles in 3 (7%) of the alcoholic hearts examined. This tends to confirm the difficulties with subjective observations if reliable on solely and completely.

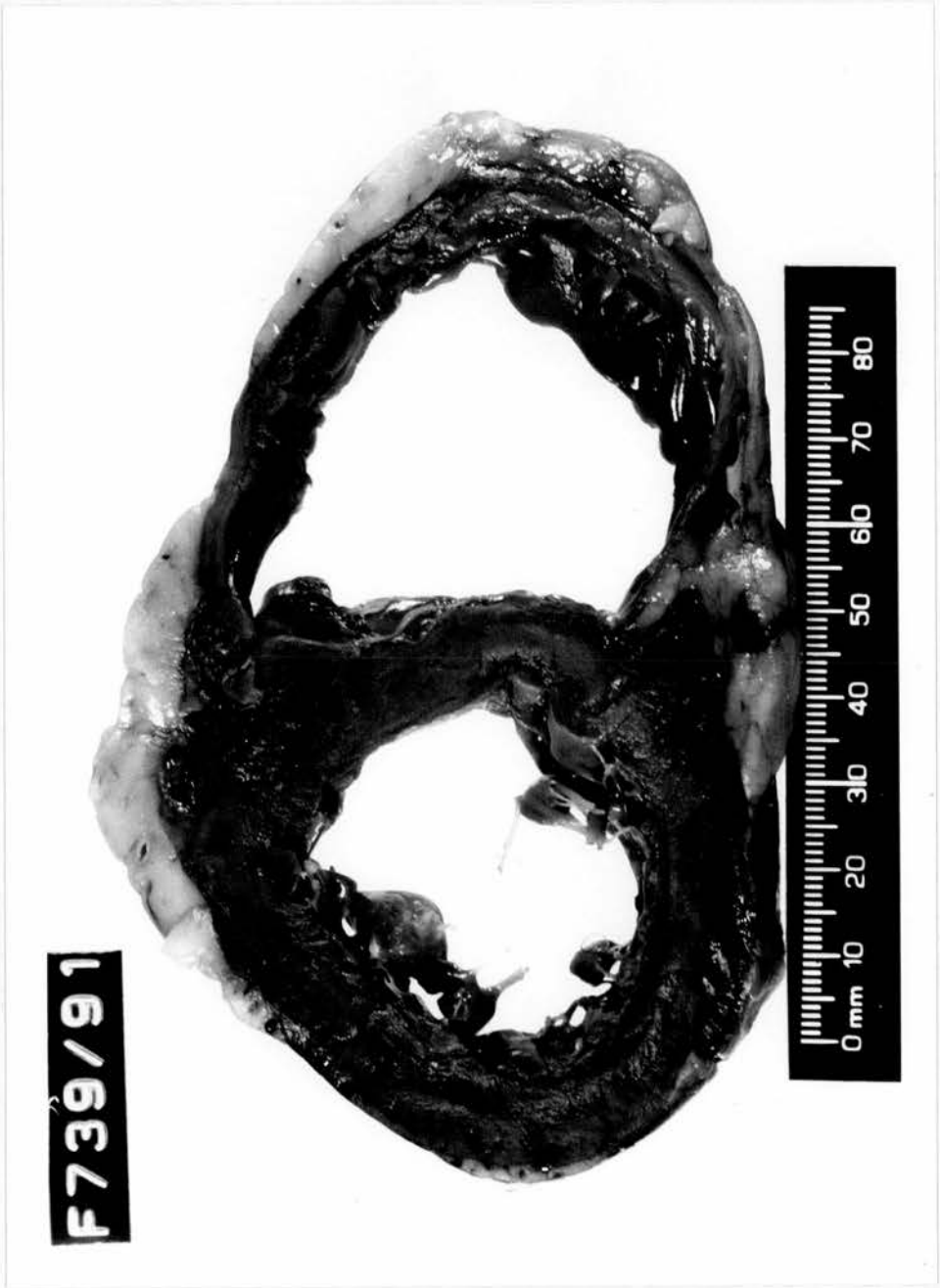


Figure 5.1 Gross transverse section of an alcoholic heart taken from the mid-point of the ventricles showing bi-ventricular dilatation.

Figure 5.2 Gross transverse section of a normal heart at mid-point of the ventricles.

Figure 5.3 Gross transverse section of an alcoholic heart at the level of the middle 1/3rd of the heart showing dilatation of the right ventricle.

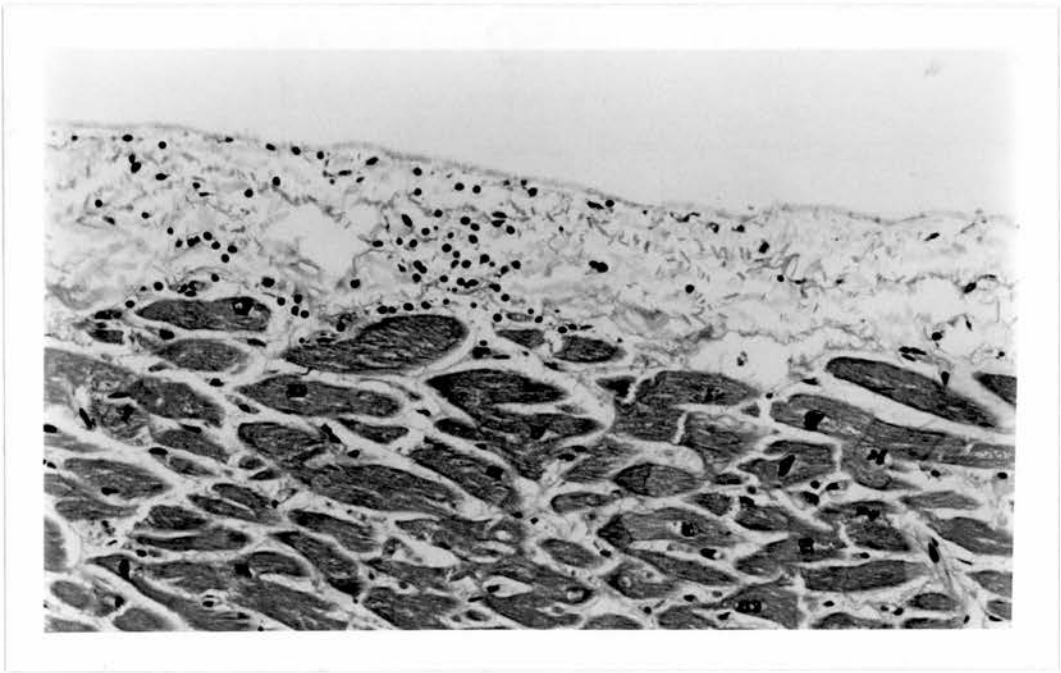


Figure 5.4 Focal chronic inflammation of the epicardium in an alcoholic (H&E x500).

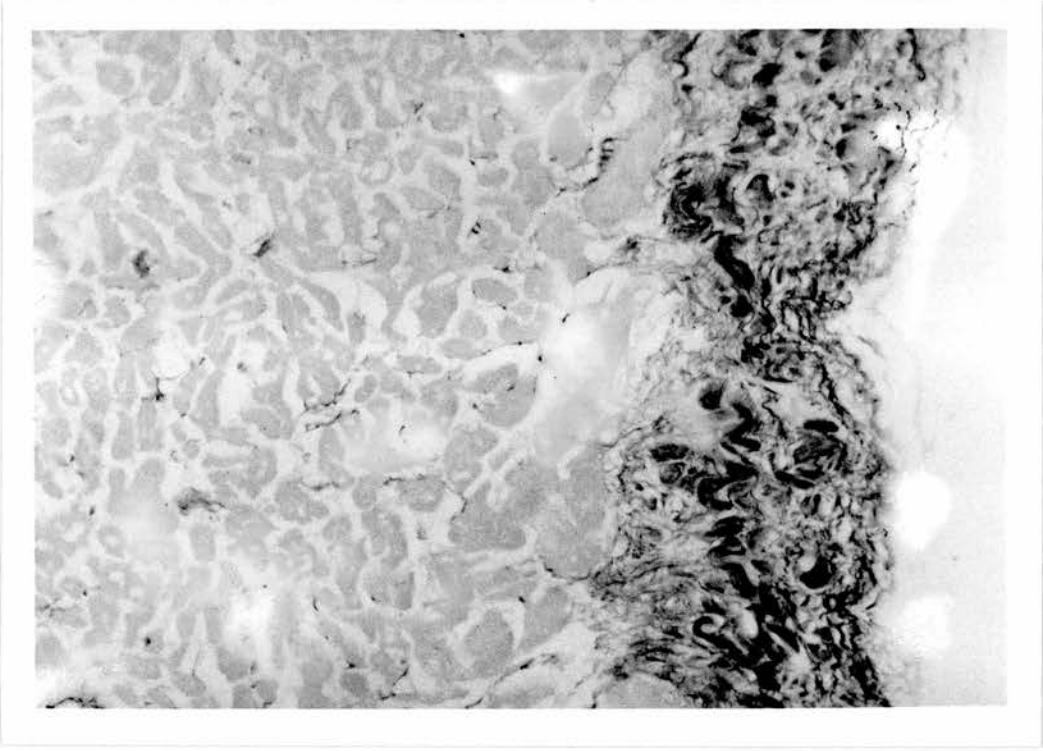


Figure 5.5 Thickening of the endocardium by fibrosis in an alcoholic (Van Geison's elastica x 250).

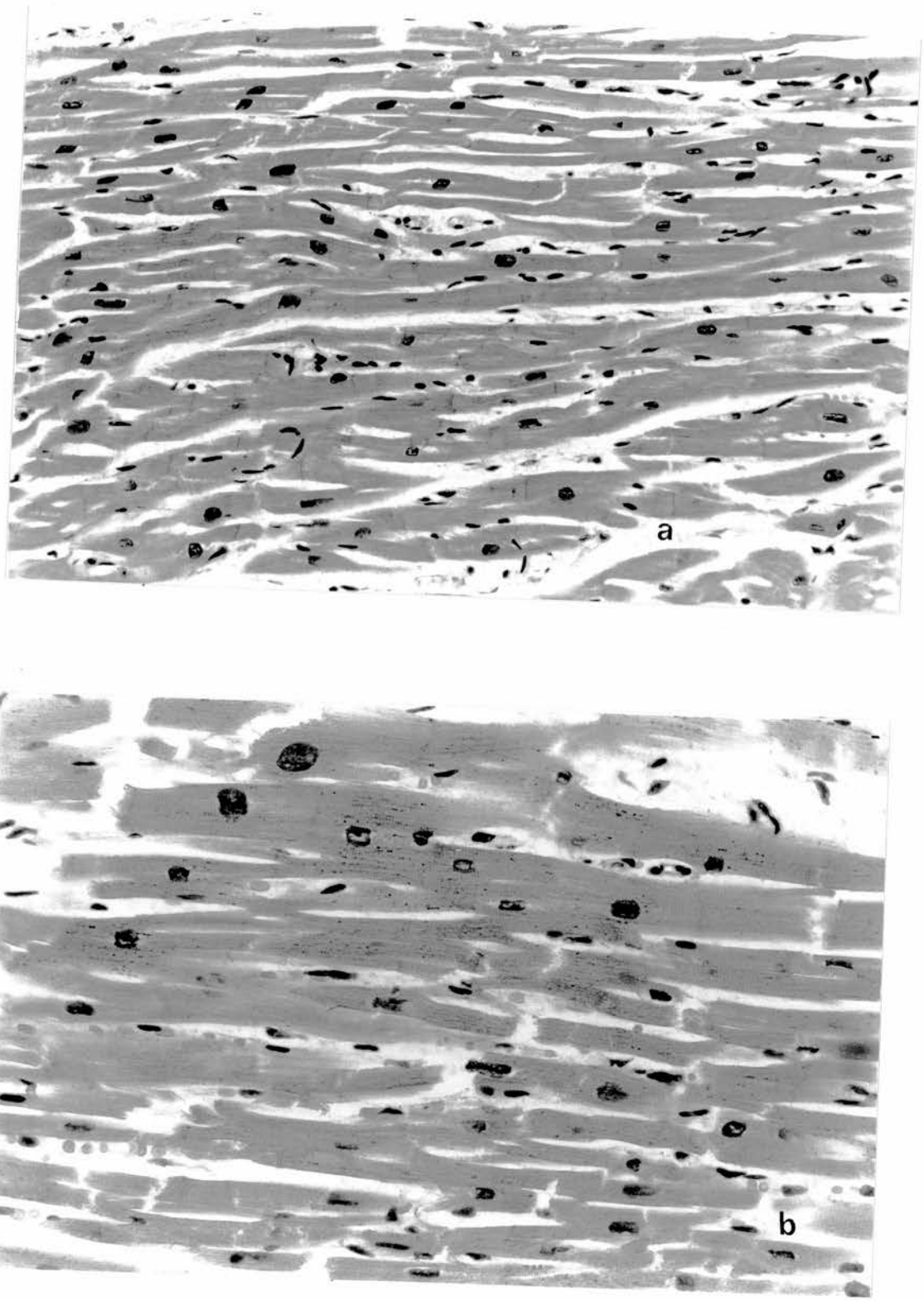
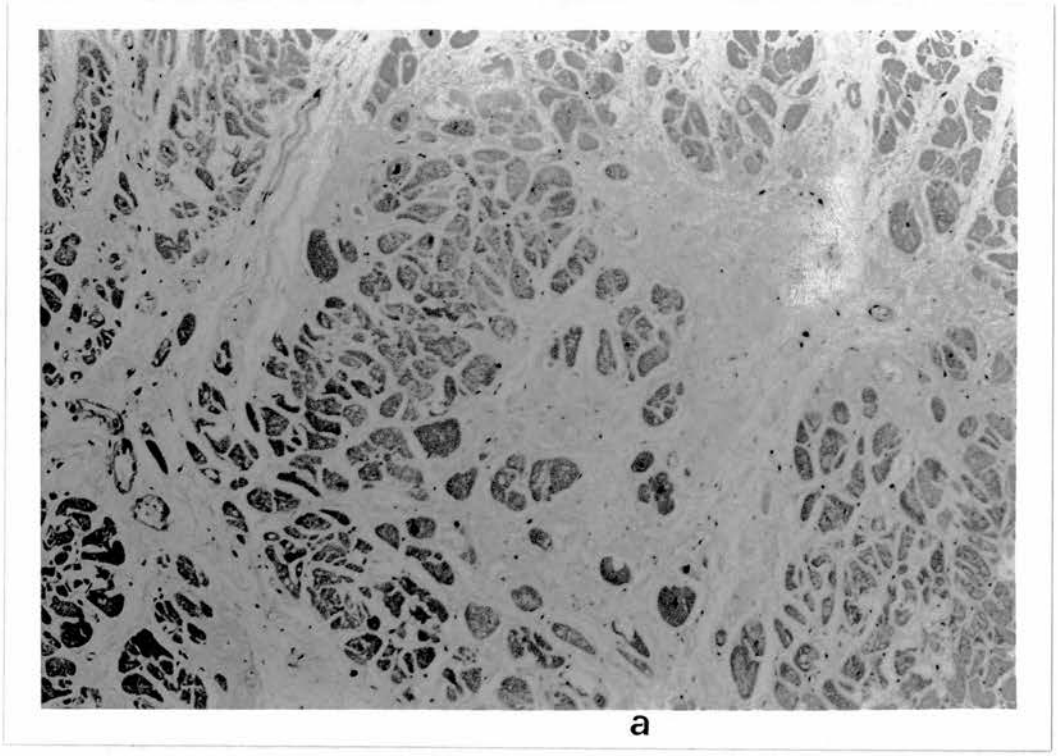


Figure 5.6 Heart microscopy sections showing enlargement of cardiac muscle fibres in an alcoholic (a) H&E x250; (b) H&E x500.

Figure 5.7 Heart microscopy section showing cross striations in a control (H&E x500).



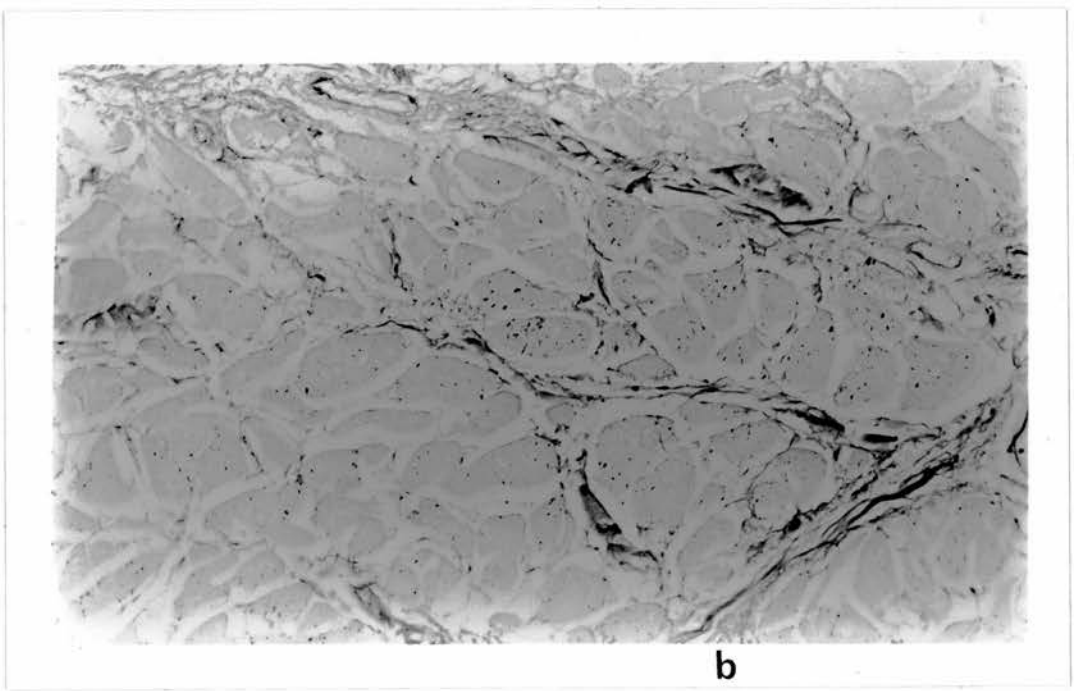


Figure 5.8 Diffuse loose inter-myocardial connective tissue in an alcoholic heart (a) H&E x250; (b) excess interstitial connective tissue (Van Geison's elastica x250).

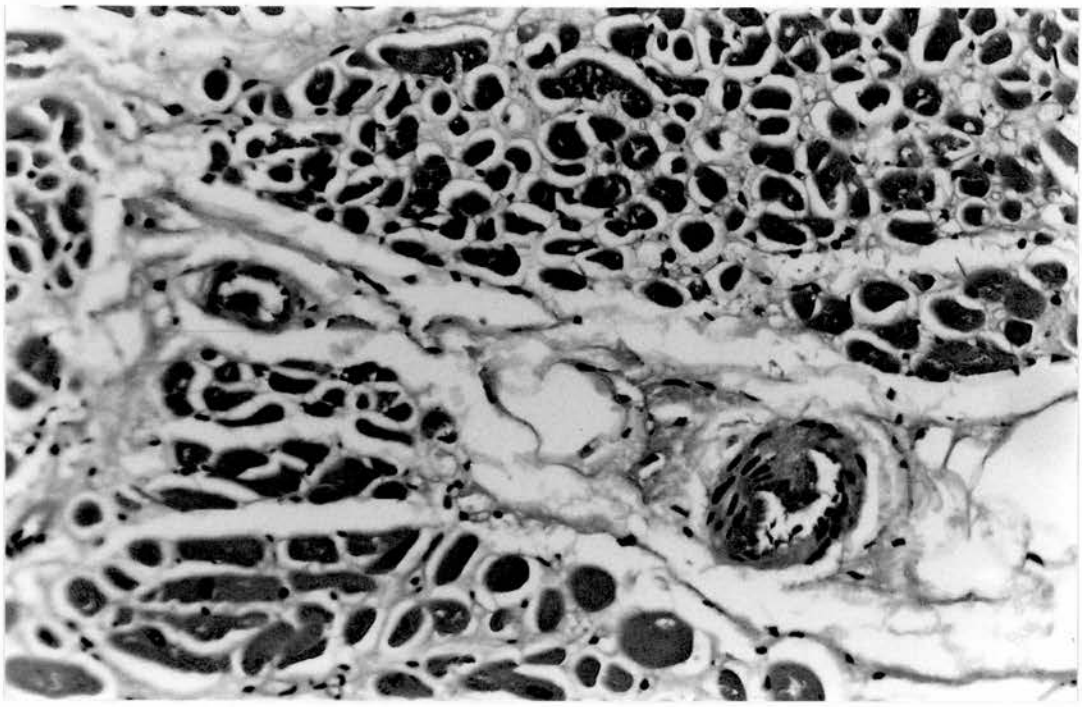


Figure 5.9a Peri-myocyte fibrosis in an alcoholic (H&E x250).

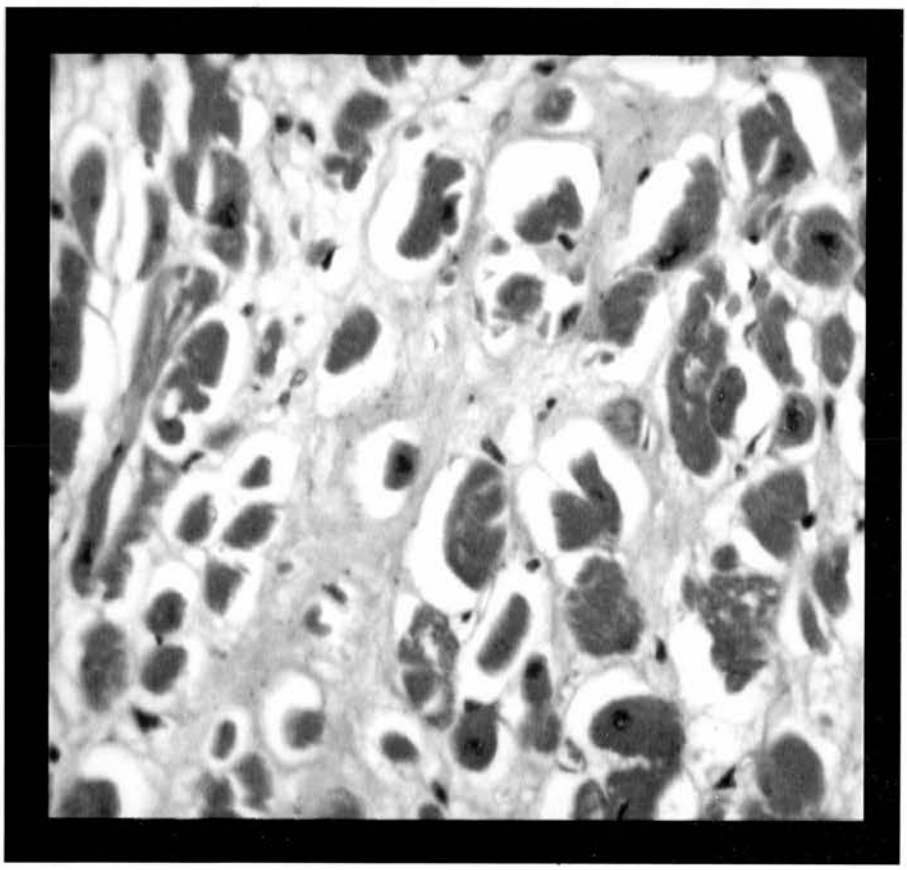
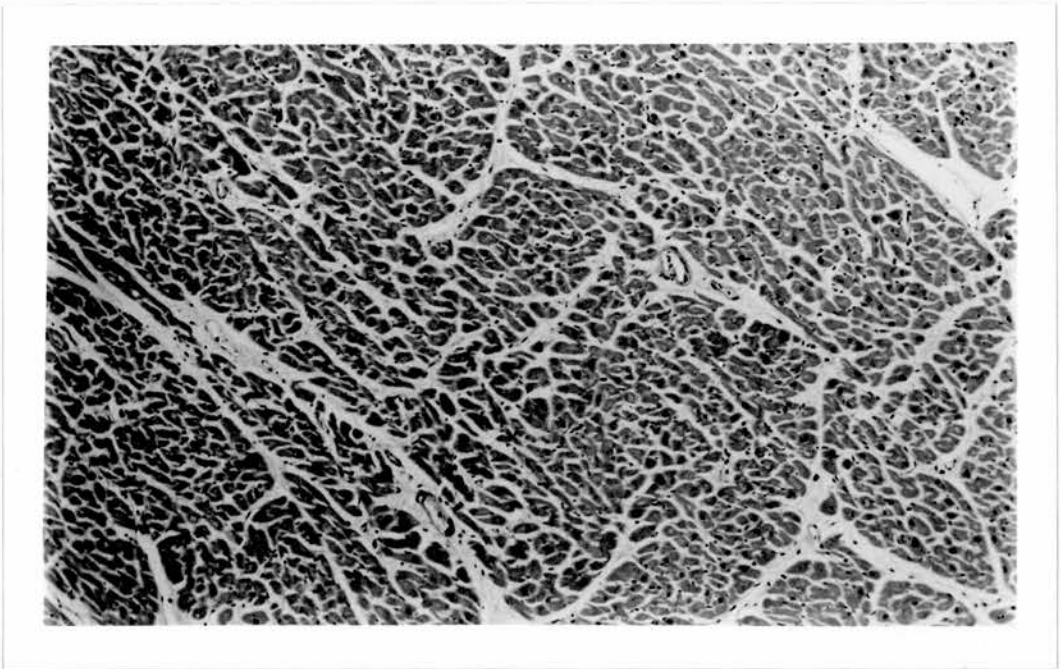
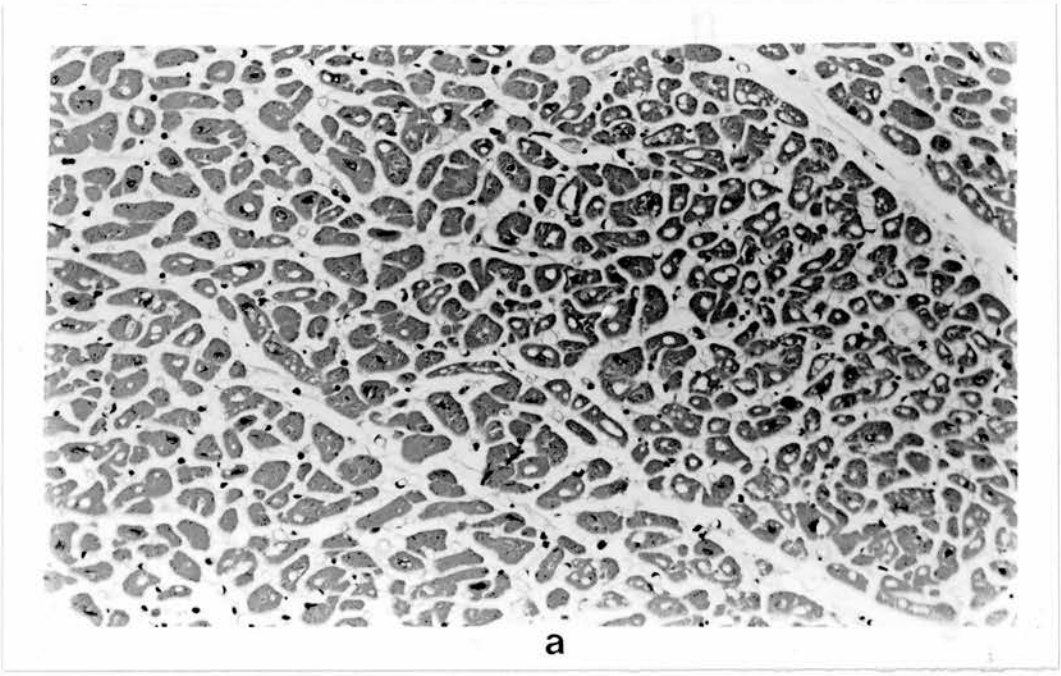


Figure 5.9b Peri-myocyte fibrosis in an alcoholic (H&E x250 polaroid photograph taken from VDU of IBAS).



5.10 Heart microscopy section from a non-alcoholic control (H&E x125).



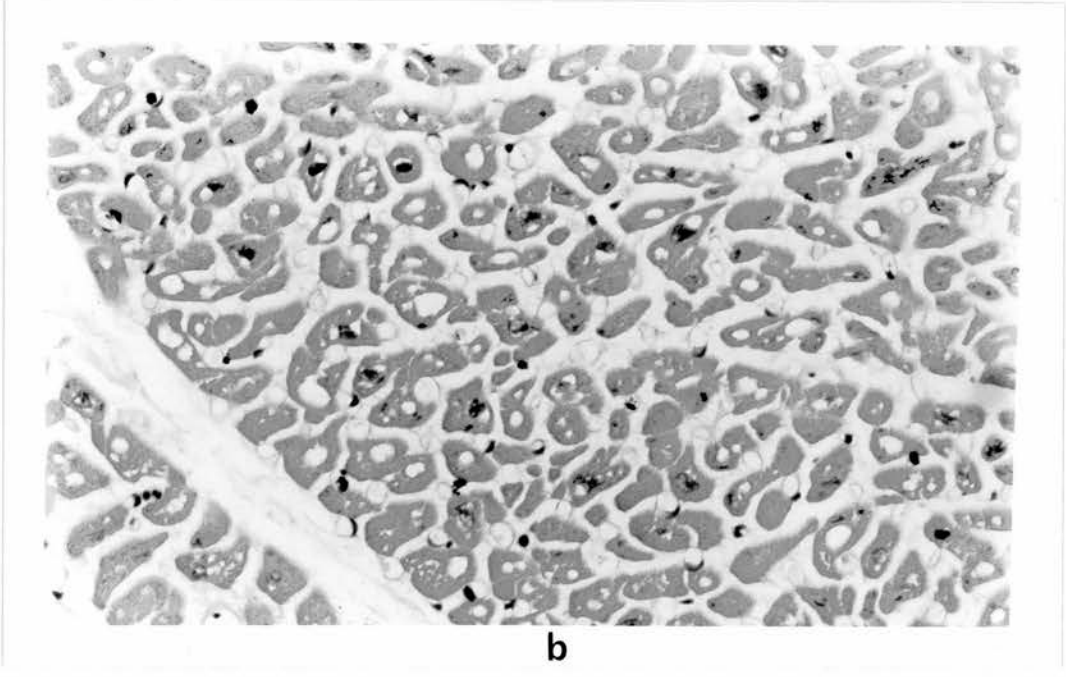


Figure 5.11 Cytoplasmic vacuolization in an alcoholic (a) H&E x250; (b) H&E x500.

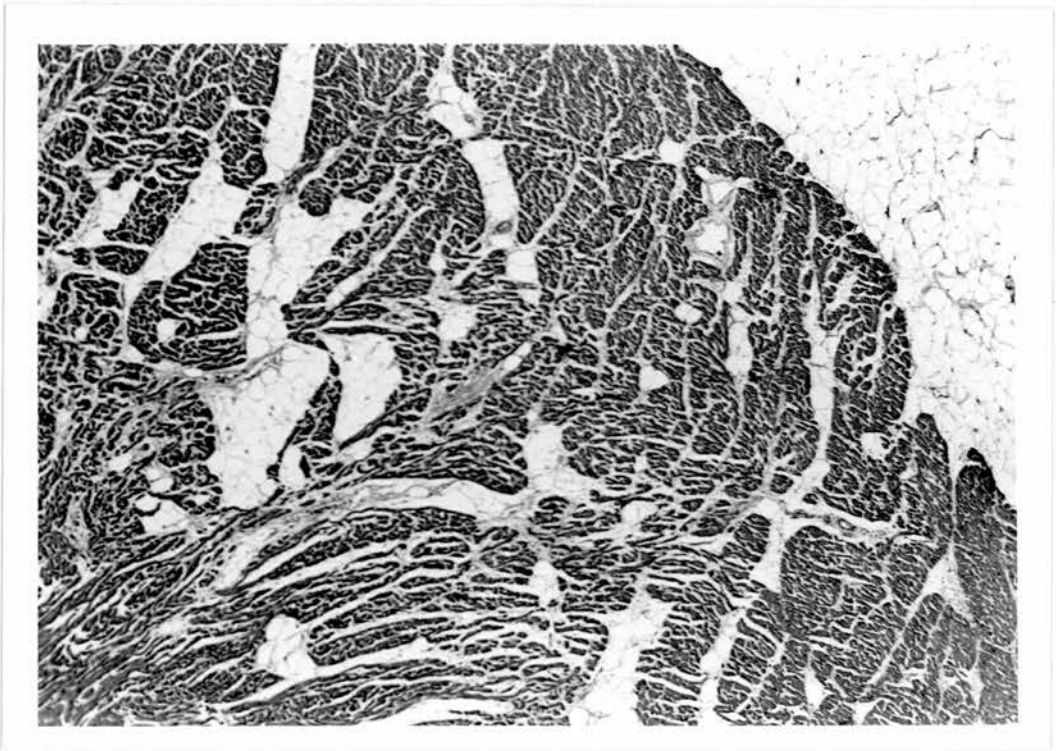
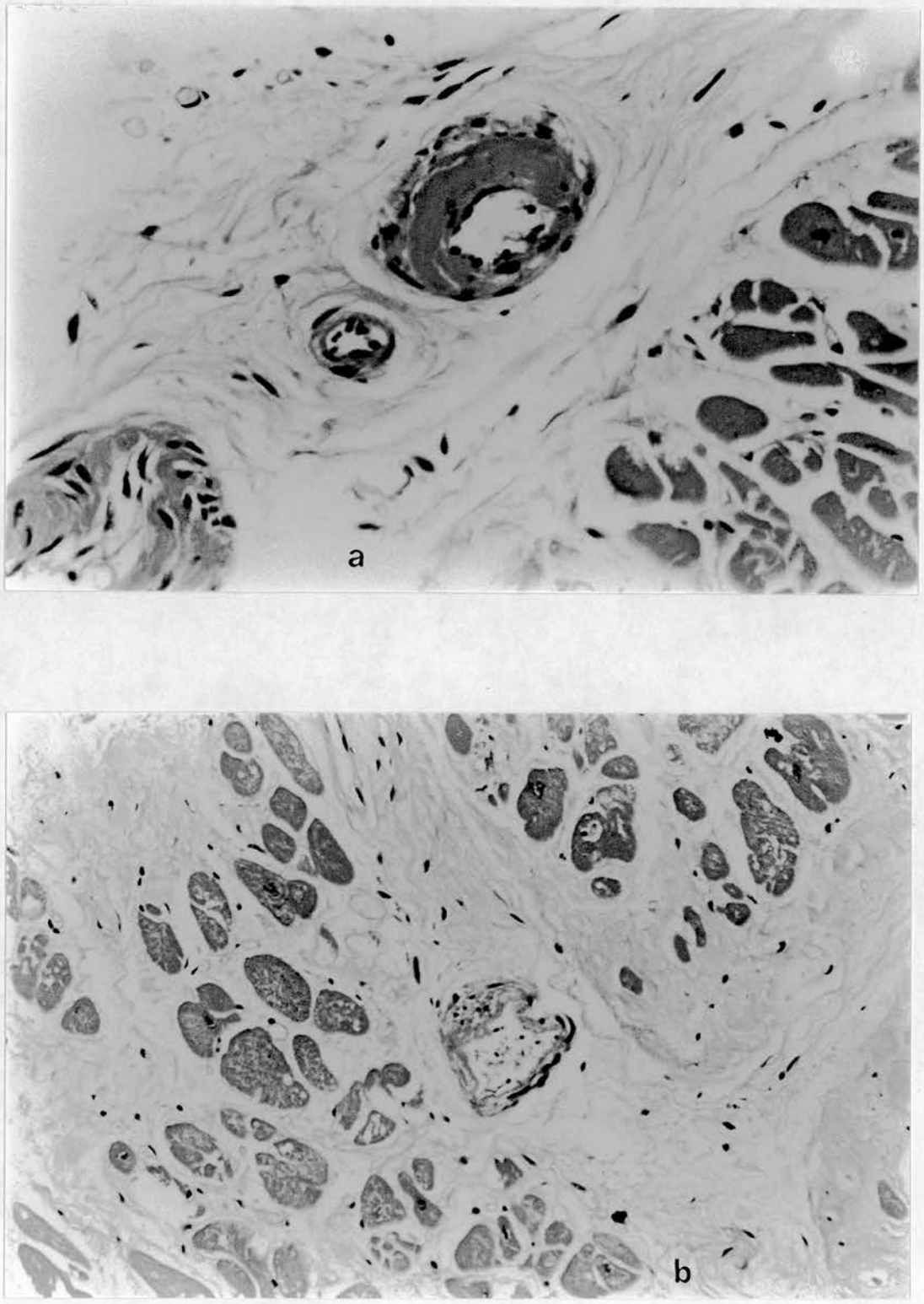
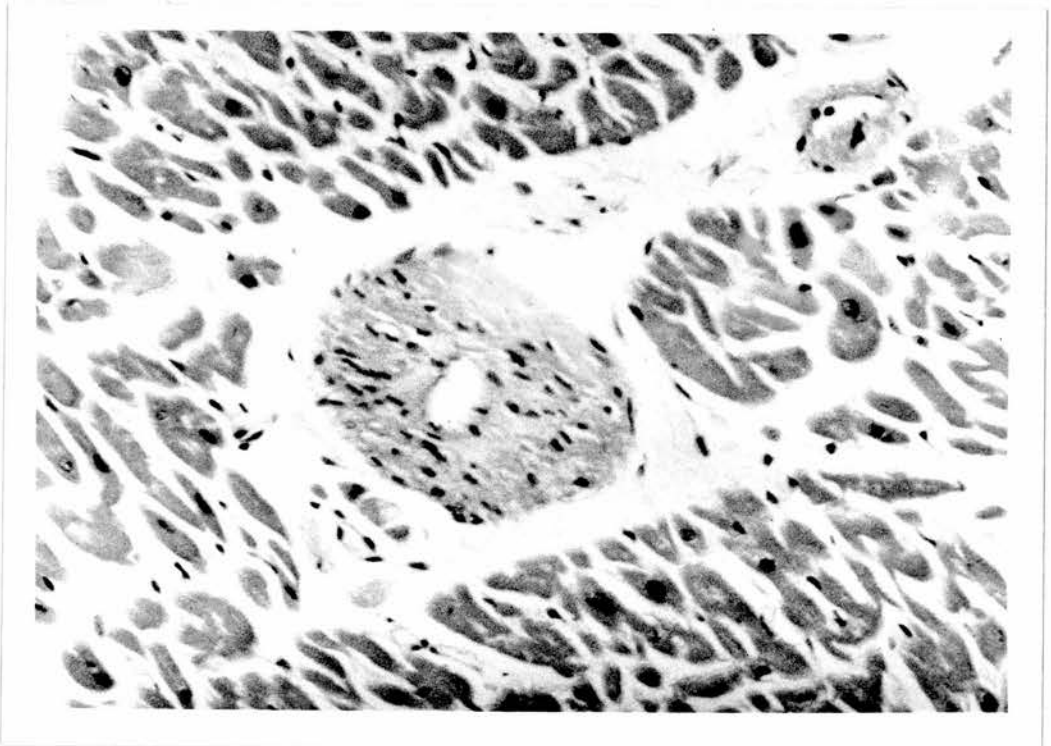


Figure 5.12 Subpericardial infiltration of the myocardium by fat in an alcoholic (H&E x125).



5.13 Excess peri-vascular connective tissue in alcoholics (a) H&E x500; (b) H&E x500.



5.14 Thickening of the medial layer of a blood vessel in an alcoholic (H&E x250).

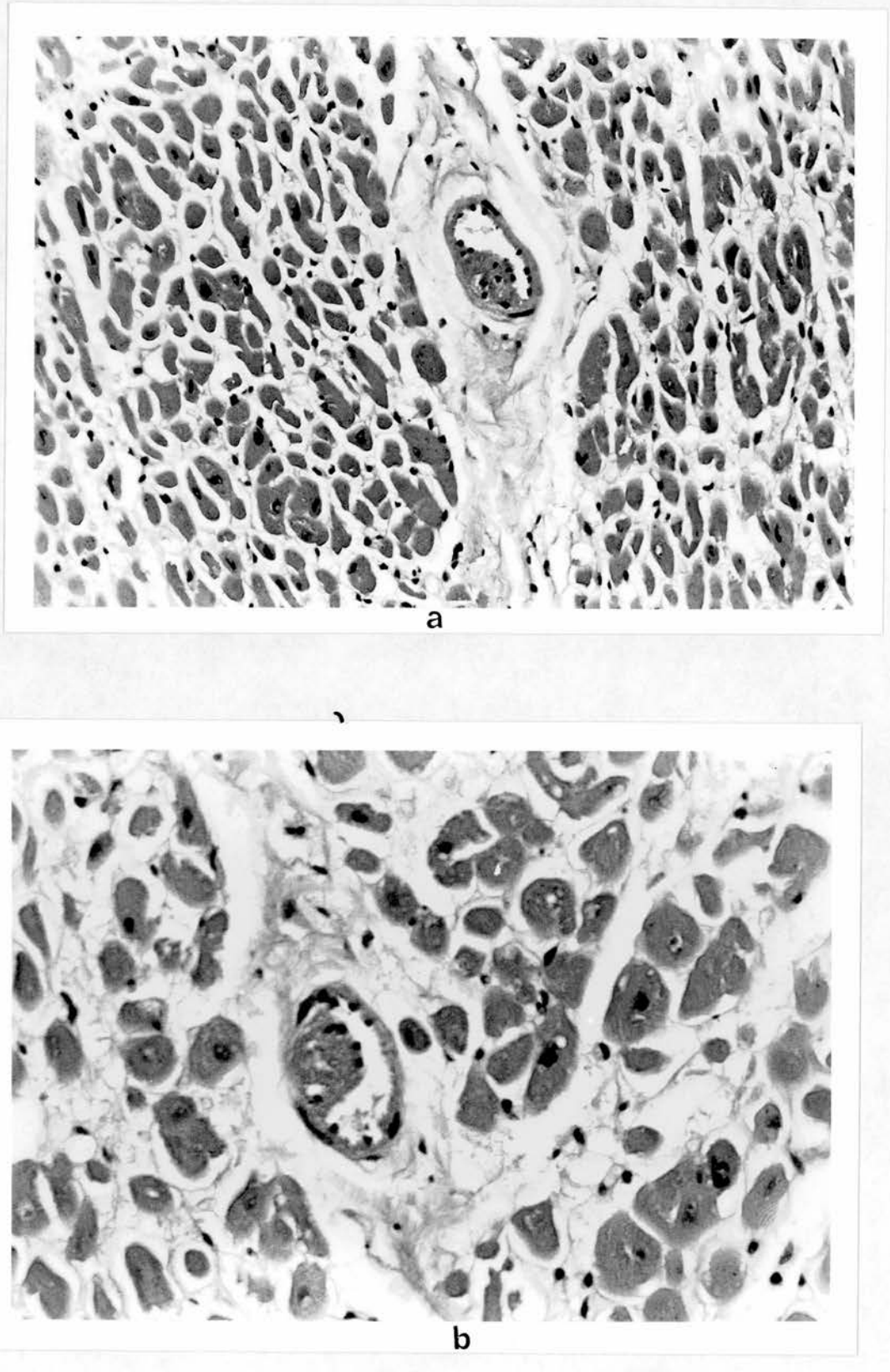


Figure 5.15 Subendothelial "hump" in alcoholics (a) H&E x250; (b) H&E x500.

Chapter 6

Results of Muscle, Connective Tissue and Nucleus Measurements

Introduction

The assessment of the individual contribution of muscle and connective tissue to the composition of the heart in alcoholics was one of the principal matters which the study wished to address. Comparisons were made between the means of the variables which were morphometrically measured; these included the area and diameter of cardiac muscle fibres, the area which accounted for in the heart by connective tissue and the nuclear diameter and area measurements. The means and standard deviations (sd) are as shown in the following tables. In these comparisons between alcoholics and controls the P-values are given using the two-sample t-test statistical analysis.

6.1 Fixation and Sectioning Artefacts

A phenomenon that can be observed on microscopy of any tissue section and particularly the heart is the presence of spaces in between the stained components of the section. The "empty space" within a section is by and large the consequence of two factors: 1. artefacts can occur in fixation and in tissue cutting and also which are likely to occur on sectioning where tissues of different densities are pulled apart though such tissues do not show this arrangement 'in vivo', and 2. the presence of fluid due to oedema and in the absence of any cause for tissue swelling due to postmortem transudation from vessels as a passive phenomenon.

The percentage "empty space" was calculated (as shown in chapter 3) to establish whether the controls and the alcoholic hearts were identical in the distribution and content of "empty space" and particularly that the latter showed no excess of artefactual spaces in them which would have to be taken specifically into account and corrected for if that were to be the case.

Table 6.1.1: Mean (sd) percentage of "empty space" with two-sample t-test statistics for group difference.

Measurement	**Region**	**Alcoholics**	**Controls**	**Significance**
Percent empty space				
	B	40.87 (4.63)	43.82 (3.88)	P<0.01
	C	39.71 (3.88)	42.00 (4.22)	P<0.05
	D	39.48 (3.80)	42.43 (3.91)	P<0.01
	E	40.15 (4.36)	42.28 (3.85)	P<0.05
	F	41.10 (4.93)	42.67 (3.79)	NS

Legend for table 6.1.1 and subsequent tables: B= right ventricle; C= anterior wall of the left ventricle; D= lateral wall of the left ventricle; E= septum; F= posterior wall of the left ventricle

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The percentage empty space was decreased in most of the alcoholic hearts as compared with the controls in this study. Figure 6.1.1 shows that alcoholics tend to have smaller areas of empty space than controls. With the exception of the measurements taken from the sections of the posterior left ventricular wall, it is shown that alcoholic hearts in this series have much fewer 'spaces' than in the controls which could be interpreted as artefact .

It is however surprising to note that with this interactive semiautomated method of measurement about two fifths of the area in a tissue section can be observed to be blank. This percentage emptiness in any particular section is almost a constant value in all the sites from which tissue sections were produced.

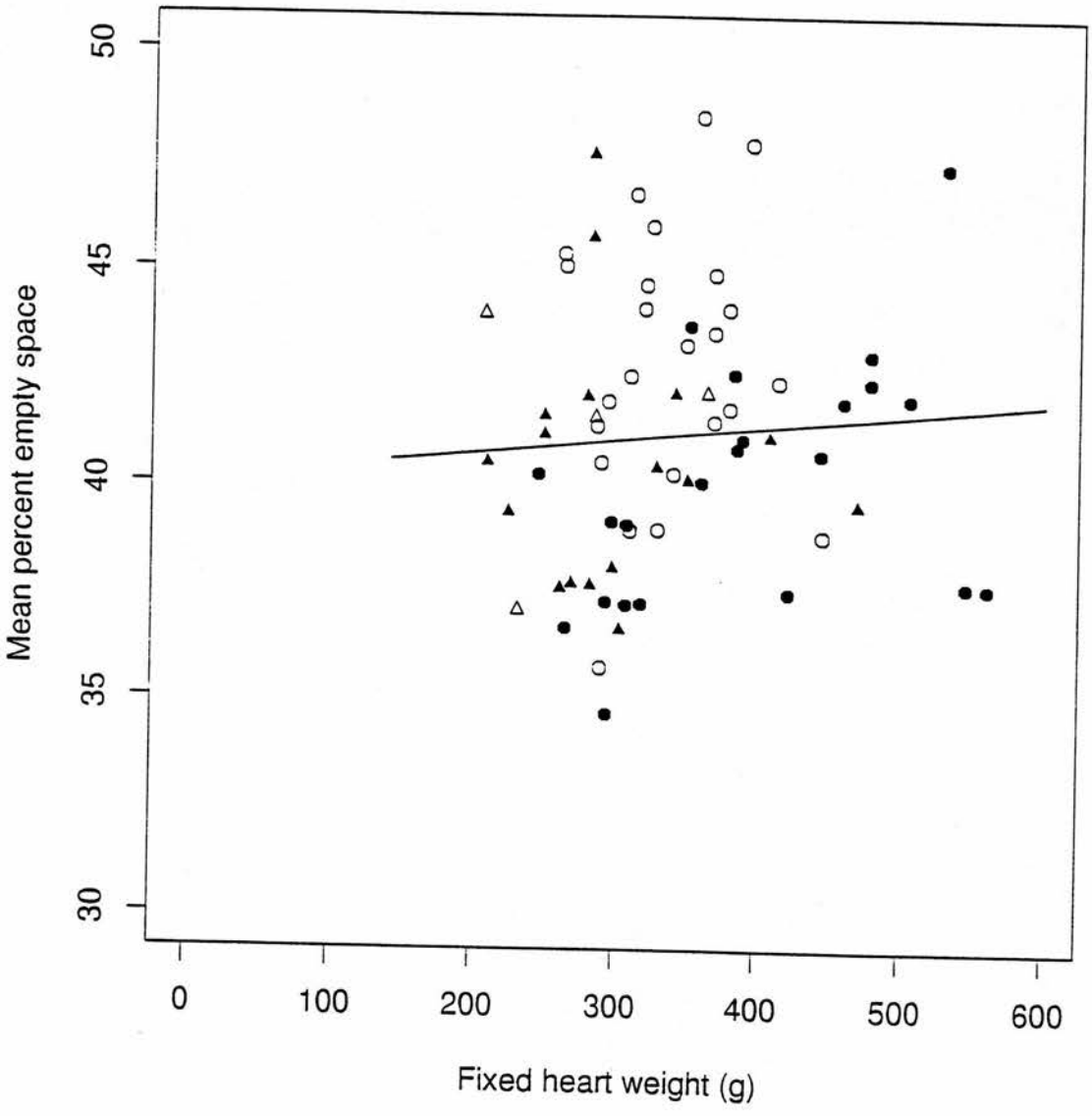

Figure 6.1.1 Plot of mean "percentage empty space" against fixed heart weight.

6.2 Measured Area of Muscle and Connective Tissue

Having shown that the two groups being measured are comparable in terms of potential artefactual non-contributory component, the relationship between the muscle and connective tissue components of the myocardium was assessed with the aim of finding out whether there is an excess of connective tissue in hearts from alcoholics. This was done by measurement of the actual area in the tissue sections which was occupied by muscle fibres and comparing this with the area occupied by connective tissues also specifically measured, having positively selected these components in the measuring procedures and obtaining absolute figures for areas of identical overall dimension in these tissue sections. This further excludes any discrepancy which would be accounted for by artefacts in the production of histological sections. The results with comparisons between the two study groups are shown in table 6.2.1.

Table 6.2.1: Measured areas containing mean (sd) muscle and connective tissue with significance test.

Measurement (μm^2)	**Region**	**Alcoholics**	**Controls**	**Significance**
Area of muscle				
	B	49.52 (5.76)	50.79 (4.64)	NS
	C	50.62 (4.66)	52.97 (4.51)	P<0.05
	D	50.76 (4.57)	52.24 (4.41)	NS
	E	49.67 (5.10)	52.31 (4.91)	P<0.05
	F	49.74 (4.20)	52.07 (4.40)	P<0.05
Area of connective tissue				
	B	9.53 (2.96)	5.39 (1.54)	P<0.001
	C	9.63 (2.47)	5.03 (1.26)	P<0.001
	D	9.75 (2.79)	5.33 (1.55)	P<0.001
	E	10.10 (2.98)	5.41 (2.18)	P<0.001
	F	9.11 (2.40)	5.26 (1.40)	P<0.001

In most of the cardiac anatomical regions which were studied, the areas occupied by muscle fibres were lower in alcoholics than in controls, while correspondingly the measured areas of connective tissue were much higher in alcoholics. Thus the actual area in the tissue sections which was comprised of connective tissue was greatly increased in alcoholics indicating that in alcoholic hearts there is an excess of connective tissue within the myocardium. The results are also illustrated in figure 6.2.1, which shows that alcoholics have much higher areas of connective tissue than controls.

It may be possible that some discrepancy could have arisen because of the random selection of the fields in which the measurements were taken with the possibility that non-representative fields might have been selected. If such bias had been present the potential for it was essentially identical in all the regions measured in both groups given that the same procedure was utilised throughout. Furthermore, a preliminary microscopical assessment of each of the tisssue sections directed the measurement to areas which were likely to be better representative of the entire section .

This alteration in the ratio of muscle to connective tissue was relatively constant throughout the entire left ventricle but was also identifiable in the sections taken from the right ventricular wall suggesting that the process by which this excess of connective tissue has been produced in the heart as a consequence of abuse of alcohol is affecting the myocardium throughout both ventricles in more or less uniform fashion.

As a further confirmation of this finding a comparison was made between the percentage of connective tissue in the hearts taken from the chronic alcoholics and controls (see chapter 3). The results which for each individual region measured are shown in table 6.2.2.

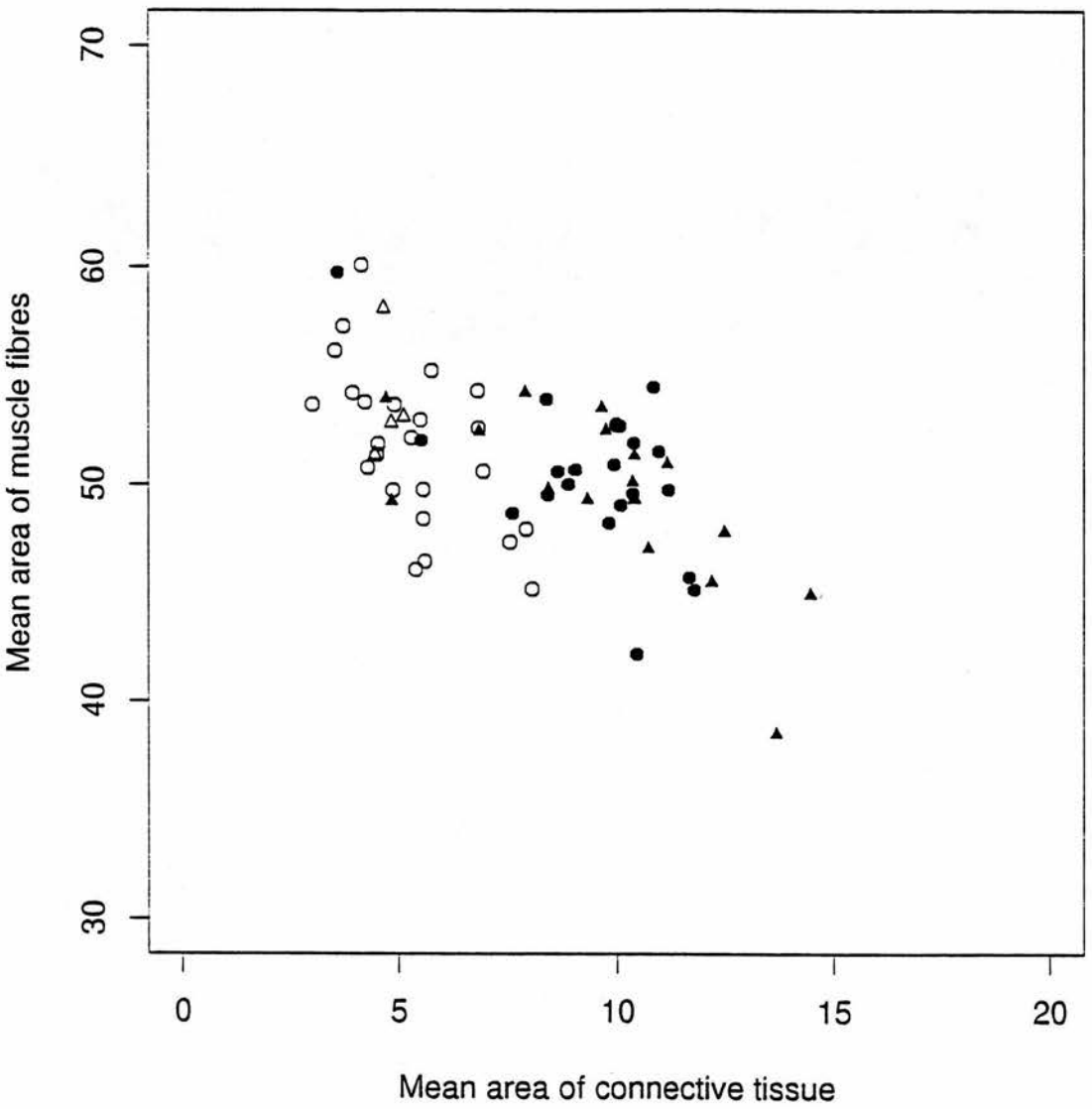

Figure 6.2.1 Plot of mean area of muscle fibres against mean area of connective tissue.

Table 6.2.2: Mean (sd) percentage connective tissue with two sample t-test statistics for group difference.

Measurement	Region	Alcoholics	Controls	Significance
% connective tissue				
	B	16.28 (5.67)	9.70 (3.09)	P<0.001
	C	16.05 (4.32)	8.73 (2.31)	P<0.001
	D	16.16 (4.69)	9.33 (2.91)	P<0.001
	E	16.94 5.29)	9.48 (4.07)	P<0.001
	F	15.49 (4.20)	9.27 (2.83)	P<0.001

Identical findings were shown and the percentage connective tissue was increased significantly in right ventricle and also in all the regions of the left ventricle in the alcoholics as compared with the controls in this study.

6.3 Diameter of Muscle Fibres

In assessing the degree of myocardial fibre hypertrophy, the measured diameter of cardiac myocytes was used as a method of assessment. A fibre that becomes hypertrophied enlarges more or less uniformly in a three-dimensional fashion and although enlarged, the general configuration of a myocyte is known to remain identical on light and ultrastructural studies even when hypertrophied. On the basis of this assumption this measurement should provide a reasonable assessment of any enlargement of the fibre.

To assess a relatively constant point within the cell which could be identified in each individual myocyte with ease. A comparison was made between the mean of the diameters of myocytes at the level of the nucleus in the hearts taken from the chronic alcoholism patients and the controls. The statistical method of two-sample t-test was used to analyse the results (see table 6.3.1).

Table 6.3.1: Mean (sd) diameter of cardiac muscle fibres with two-sample t-test for group difference.

Measurement (μm)	**Region**	**Alcoholics**	**Controls**	**Significance**
Diameter of muscle				
	B	11.86 (1.95)	11.14 (1.17)	NS
	C	13.43 (1.09)	12.32 (0.94)	P<0.001
	D	14.41 (1.64)	13.06 (1.12)	P<0.001
	E	13.80 (1.44)	12.75 (1.12)	P<0.01
	F	13.89 (1.41)	12.77 (1.00)	P<0.001

Alcoholics showed greater diameters of muscle fibres as compared with controls and this feature could be shown throughout all regions of the left ventricle. (see table 6.3.1). Figure 6.3.1 shows that alcoholics have greater diameters of muscle fibres compared with controls. The exception was that there was no statistically significant variation in the myocyte diameters for right ventricle in alcoholics compared with the controls. This indicates a generalised left ventricular myocyte hypertrophy.

6.4 Nuclei of Cardiac Muscle Fibres

As a further assessment of the degree of hypertrophy present, nuclear area and diameter were measured given that nuclear enlargement is an invariable component of the pathological processes that take place in muscle hypertrophy. As a further assessment of this phenomenon the ratio of the diameter of the nucleus to that of muscle fibre was calculated (see chapter 3).

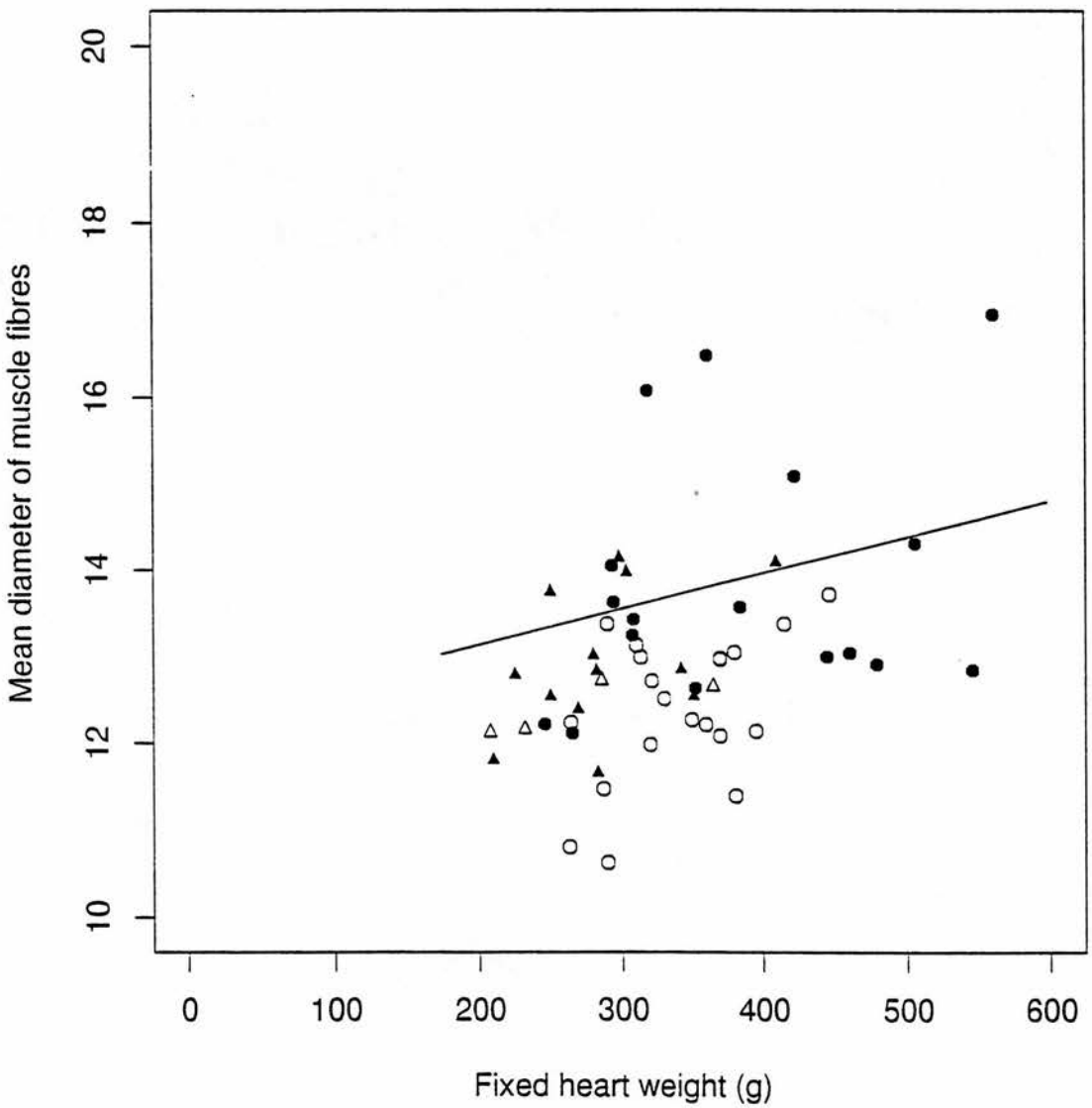

Figure 6.3.1 Plot of mean diameter of muscle fibres against fixed heart weight.

The two measurements of nuclei which were taken were the area occupied by the nucleus itself as well as its minimum diameter (see table 6.4.1 and 6.4.2).

Table 6.4.1: Mean (sd) area of nuclei of cardiac muscle fibres with two sample t-test statistics for group difference.

Measurement (μm^2)	**Region**	**Alcoholics**	**Controls**	**Significance**
Area of nucleus				
	B	35.84 (10.02)	31.73 (7.20)	NS
	C	39.62 (12.73)	31.90 (8.38)	P<0.01
	D	36.77 (7.61)	32.34 (6.28)	P<0.05
	E	36.35 (8.22)	30.46 (6.31)	P<0.01
	F	39.11 (9.64)	32.51 (8.84)	P<0.01

Table 6.4.2: Mean (sd) diameter of nuclei of cardiac muscle fibres with two sample t-test statistics for group difference.

Measurement (μm)	**Region**	**Alcoholics**	**Controls**	**Significance**
Diameter of nucleus				
	B	5.14 (0.80)	5.00 (0.53)	NS
	C	5.81 (0.83)	5.19 (0.62)	P<0.01
	D	5.64 (0.62)	5.24 (0.49)	P<0.01
	E	5.59 (0.63)	5.07 (0.62)	P<0.01
	F	5.90 (0.82)	5.30 (0.63)	P<0.01

As indicated in chapter 3, the 'relative' area of nucleus was calculated using the formula:

Relative area of nucleus=100 x (diameter of nucleus / diameter of muscle fibre)^2

Table 6.4.3: Mean (sd) 'relative' area of nuclei of cardiac muscle fibres with two sample t-test statistics for group difference.

Measurement (μm)	**Region**	**Alcoholics**	**Controls**	**Significance**
Relative area of nucleus				
	B	22.23 (9.31)	20.77 (5.15)	NS
	C	18.94 (5.39)	18.08 (4.01)	NS
	D	15.81 (4.65)	16.42 (4.34)	NS
	E	16.76 (4.28)	15.98 (3.85)	NS
	F	17.99 (4.53)	17.64 (4.14)	NS

Alcoholics showed increased areas and diameters of cardiac muscle fibre nuclei as compared with the controls in this study in all areas of the heart except, in agreement with the previous comparisons, the right ventricular measurements (see table 6.4.1 and 6.4.2). There were no significant differences in the ratio of nuclear to cytoplasmic areas, as shown in figure 6.4.1, suggesting that the muscle fibre enlargement in alcoholics involved to the same extent both the nucleus and the rest of the cell components.

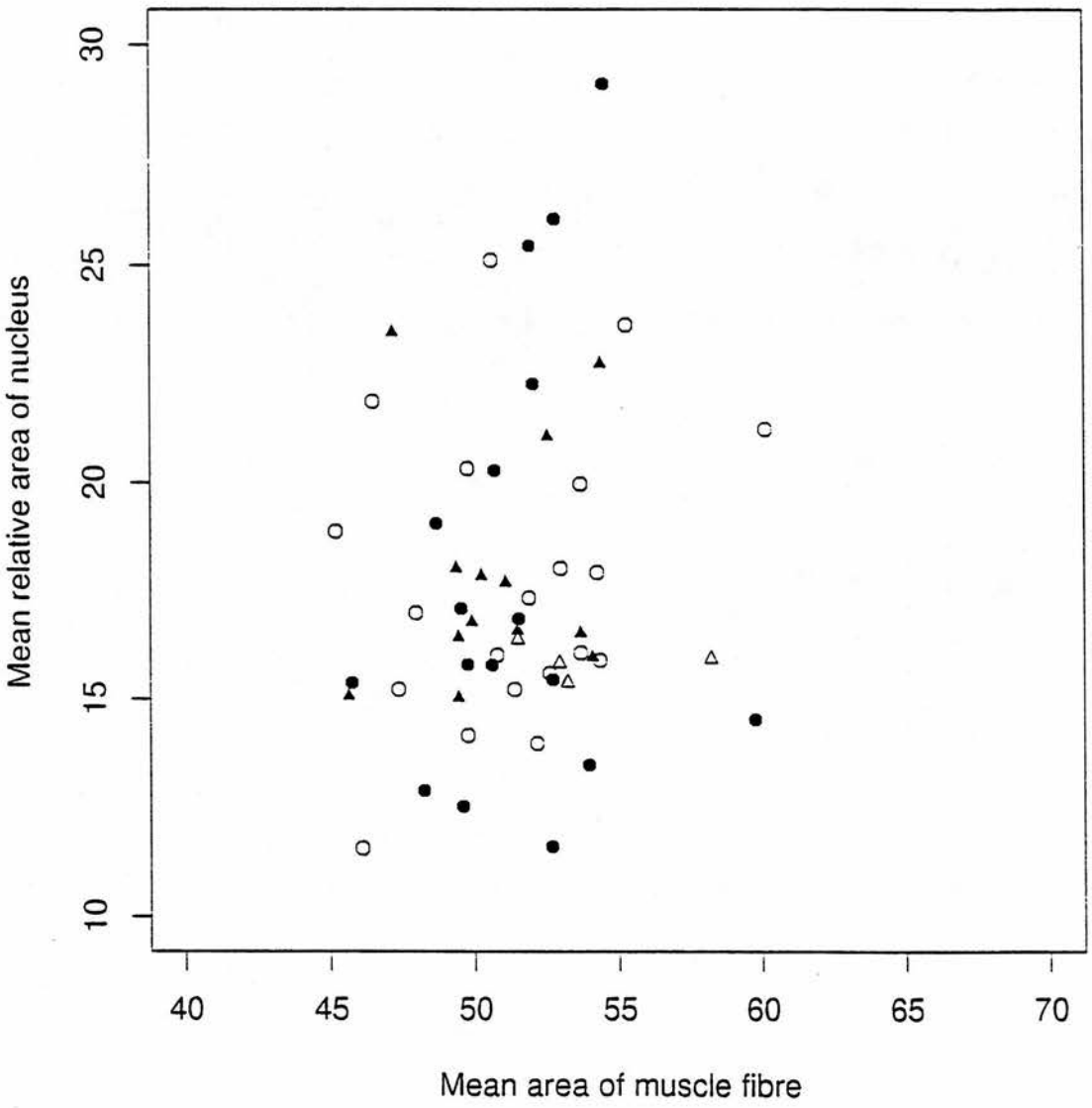

Figure 6.4.1 Mean relative area of nuclei plotted against mean area of muscle fibres.

6.5 Correlation of Measurements with Heart Weight

In an attempt to correlate the changes in the muscle fibres with heart weight, the mean area of muscle fibres from all the anatomical regions of the heart (averaged over all five regions) that was examined in this study from both the controls and the alcoholics were plotted against fixed heart weight. In figure 6.5.1 the plotted figures show that the alcoholics tend to have smaller areas occupied by muscle fibres as compared with the controls and no strong association can be demonstrated between the area occupied by muscle fibres and the fixed weight of the heart.

Similarly, as shown in figure 6.5.2, when the mean area occupied by connective tissue is taken for all the anatomical regions of the heart (averaged over all five regions) that was examined in this study from both the controls and the alcoholics and plotted against fixed heart weight, alcoholics have significantly increased areas of connective tissue as compared with the controls. However, no strong association between the connective tissue area and the fixed heart weight can be shown.

If a mean for percentage area of connective tissue is calculated for all the anatomical regions of the heart (averaged over all five regions) examined from both controls and alcoholics and these figures plotted against fixed heart weight it is shown that alcoholics have significantly increased percentage connective tissue as compared with controls, where there is a little overlap between the groups, but this finding shows no strong association with fixed weight of the heart (see figure 6.5.3).

Furthermore, as shown in figure 6.5.4, the mean diameters of muscle fibres taken from all the anatomical regions of the heart are plotted against fixed heart weight. It can be shown that the alcoholics tend to have an increased diameter of muscle fibres as compared with the controls as shown from the previous statistical analysis. The diameter of muscle fibres show some dependence on fixed heart weight (see figure 6.5.4).

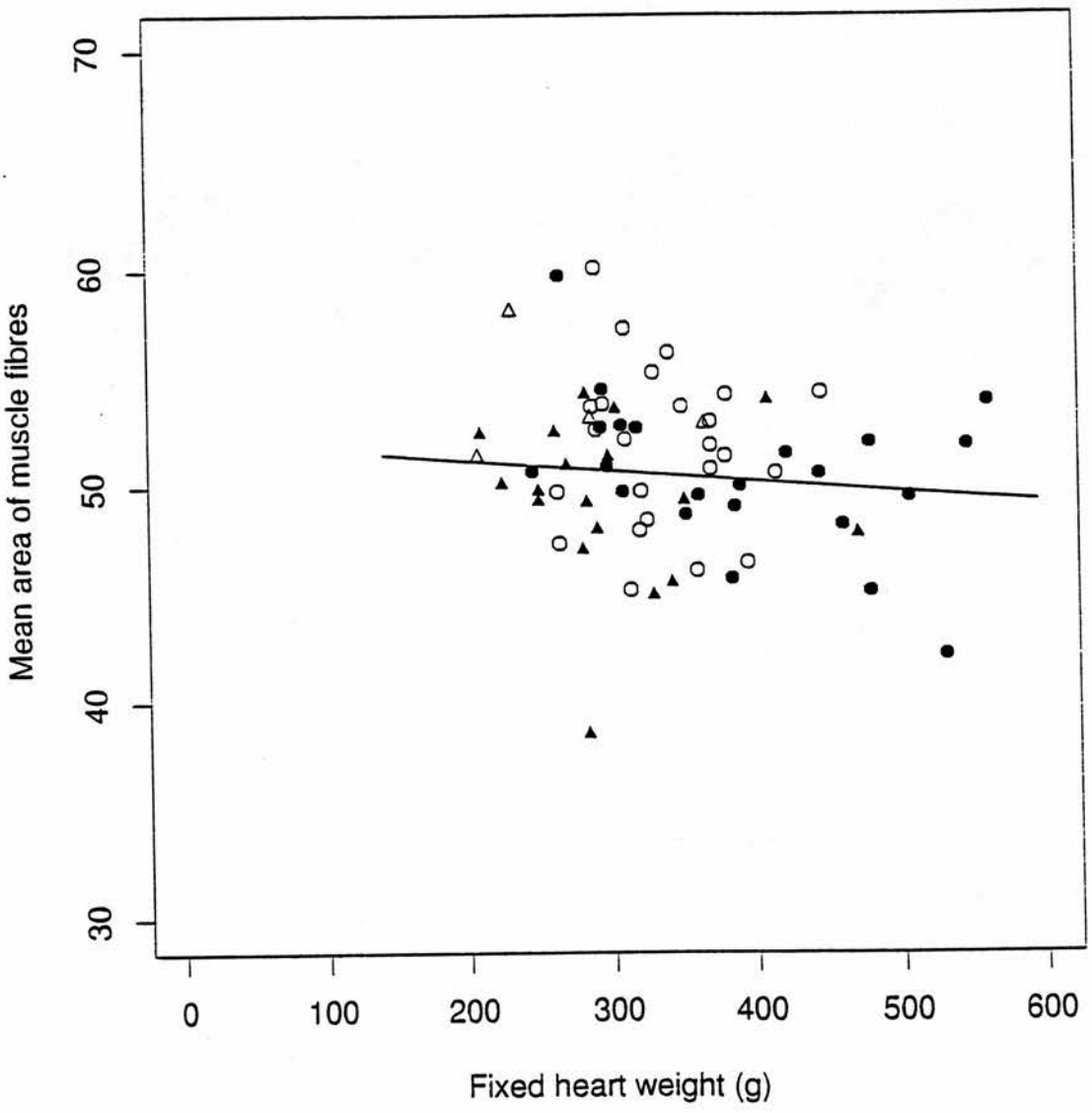

Figure 6.5.1 Mean area of muscle fibres plotted against fixed heart weight.

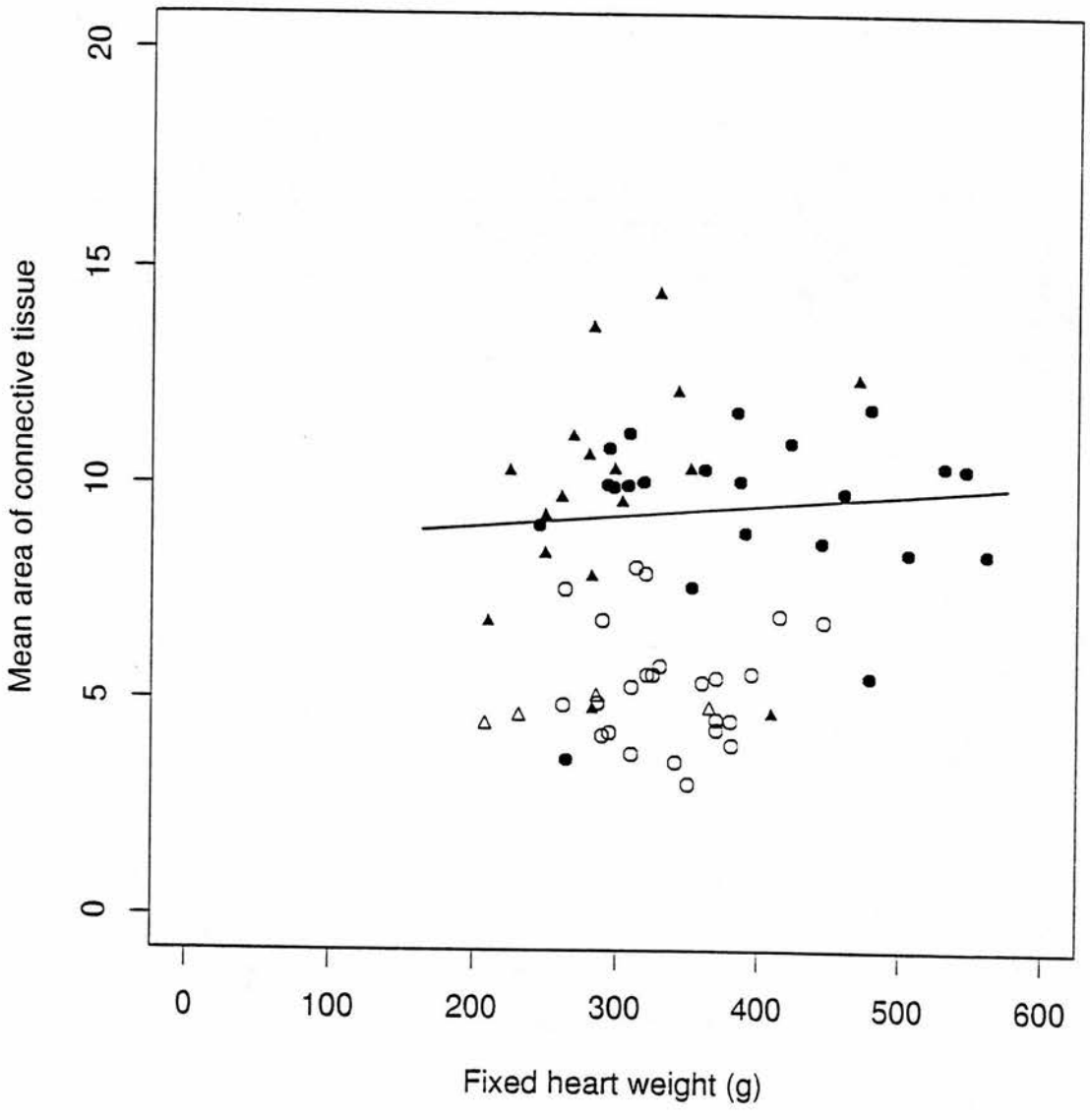

Figure 6.5.2 Plot of mean area of connective tissue against fixed heart weight.

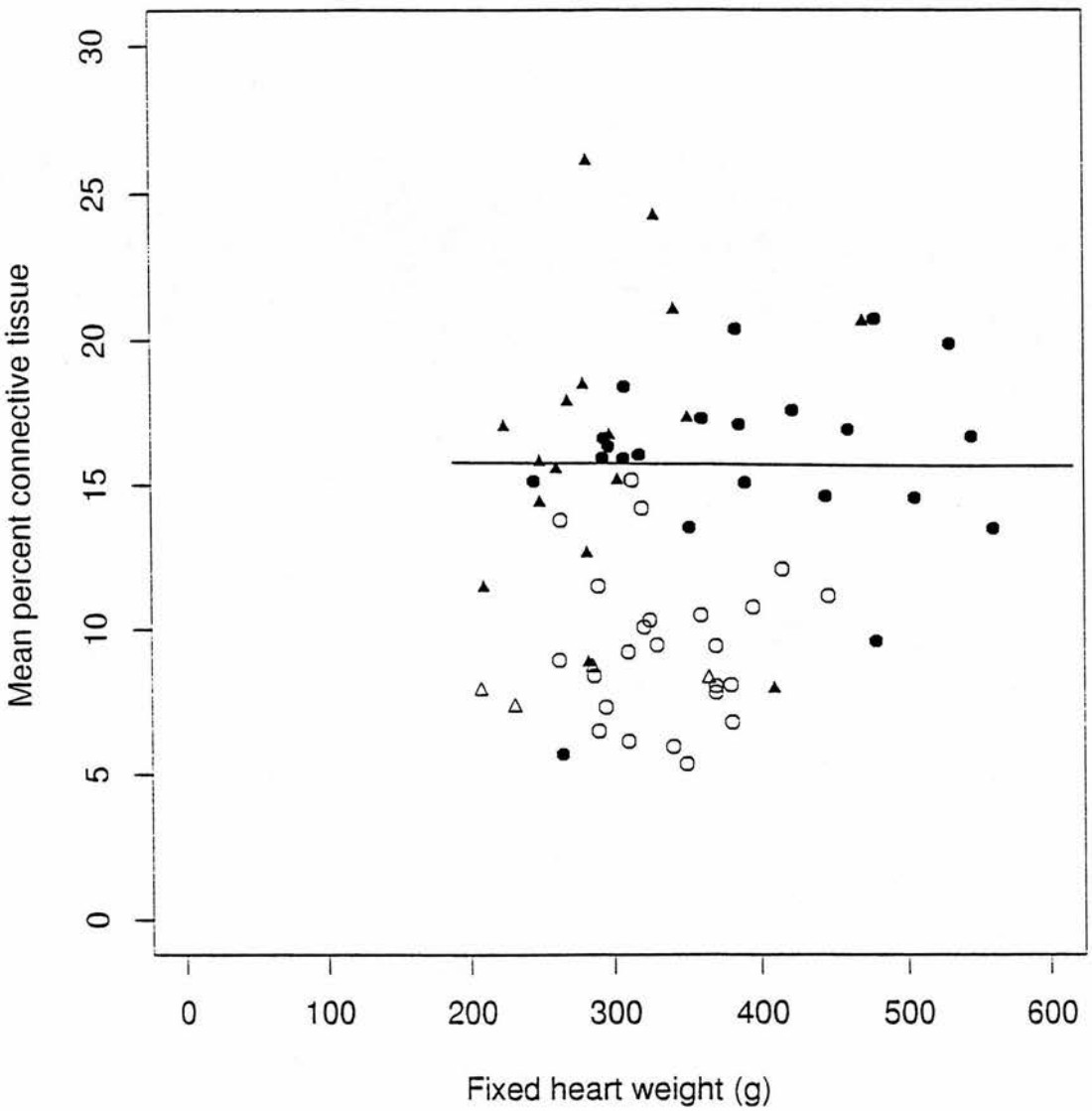

Figure 6.5.3 Mean percent connective tissue plotted against fixed heart weight.

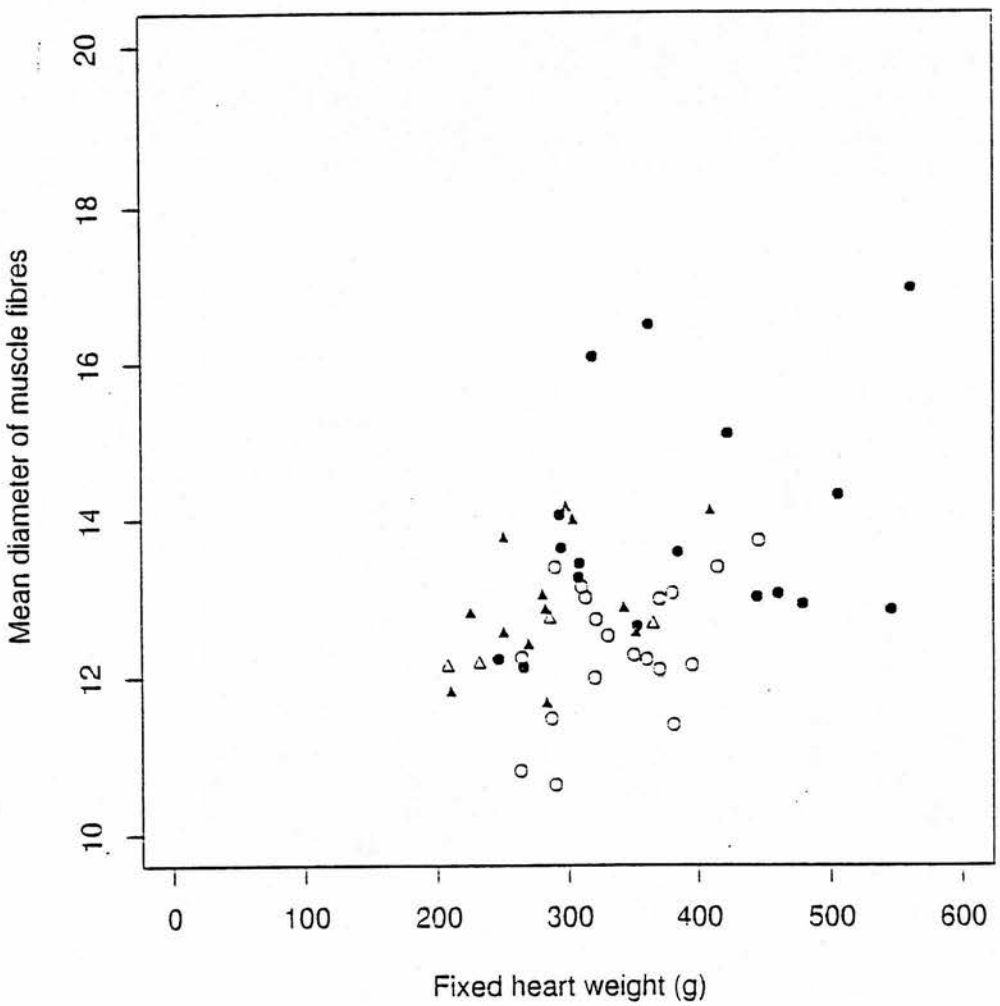

Figure 6.5.4 Mean diameter of muscle fibres plotted against fixed heart weight.

Figure 6.5.5 shows a plot for the mean area occupied by cardiac muscle fibre nuclei (averaged over all five regions of the heart) against the fixed heart weight. This demonstrates that alcoholics tend to have an increase in the area within cardiac muscle fibres occupied by the nucleus as compared with controls. The area which the nuclei comprise shows no strong association with fixed heart weight.

Similarly, in figure 6.5.6, the diameters of nuclei of cardiac muscle fibres calculated as the mean (averaged over all five regions of the heart) were examined in this study from both the controls and the alcoholics and were plotted against the fixed weight of the heart. Alcoholics tended to have an increased diameter of cardiac muscle fibre nuclei as compared with controls. The diameters of nuclei demonstrate some dependence on fixed heart weight.

When the mean 'relative area' of cardiac muscle fibre nuclei is plotted against the fixed weight of the heart it was found that the relative area of nuclei is not strongly associated with fixed heart weight, in both alcoholics and controls (see figure 6.5.7).

6.6 Comparisons of different Regions of the Left Ventricle in Controls

Pathologists have largely tended to assume that individual regions of the heart show similar or identical features in the various parameters of myocyte microscopy particularly those that have been specifically looked at in this study i.e. whichever area of the left ventricular wall that is examined the features of the individual myocytes are roughly similar. Any inter-regional variations which may actually be present, have not been considered to be so pronounced as to be specifically and accurately assessed. Given the facilities and the various measurements which were available in the control specimens in this study, these variations were looked into and specifically defined. These are listed in table 6.6.1. This was carried out in an effort to establish a suitable baseline for the studies on alcoholic hearts.

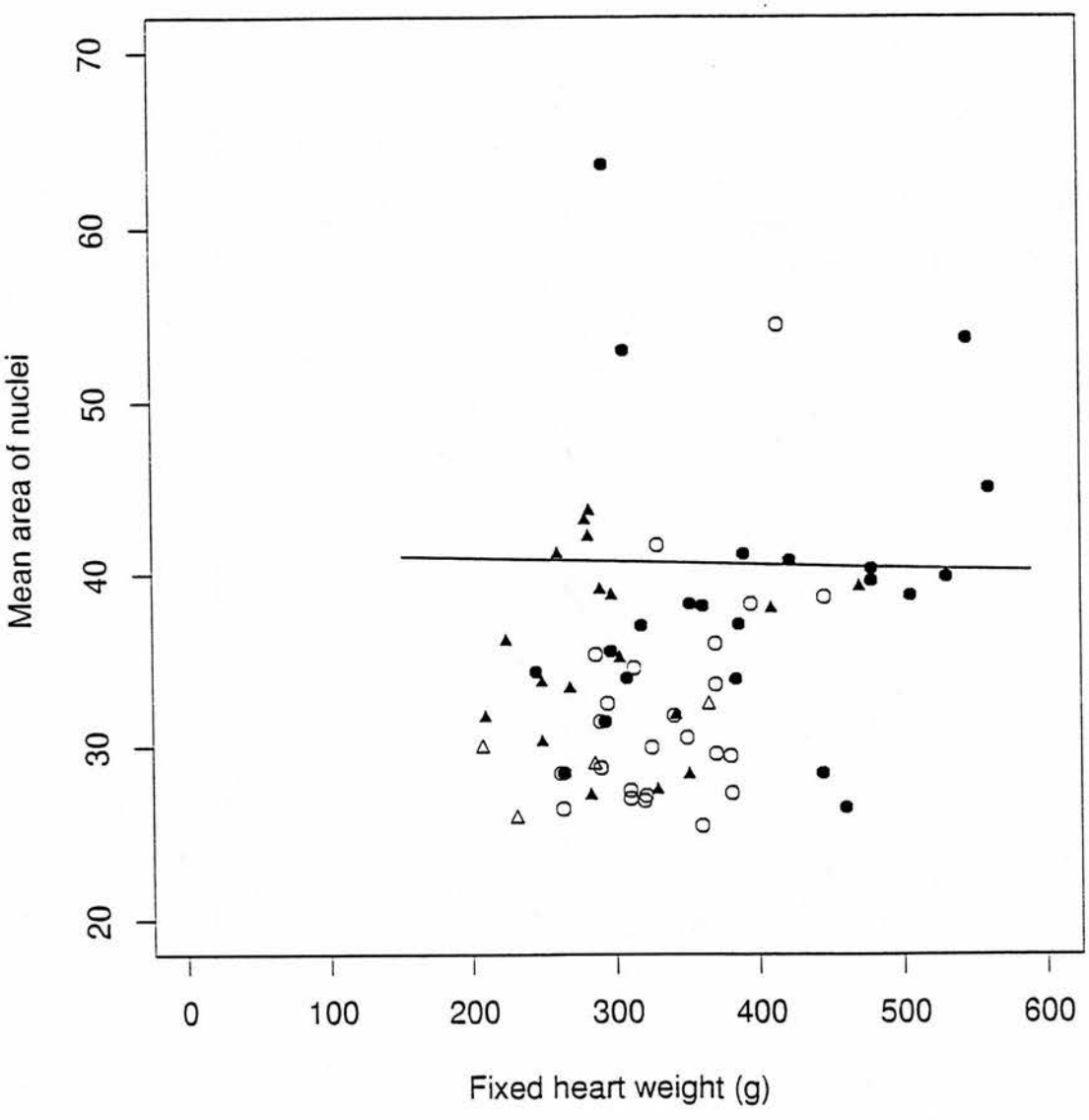

Figure 6.5.5 Plot of mean area of nuclei against fixed heart weight.

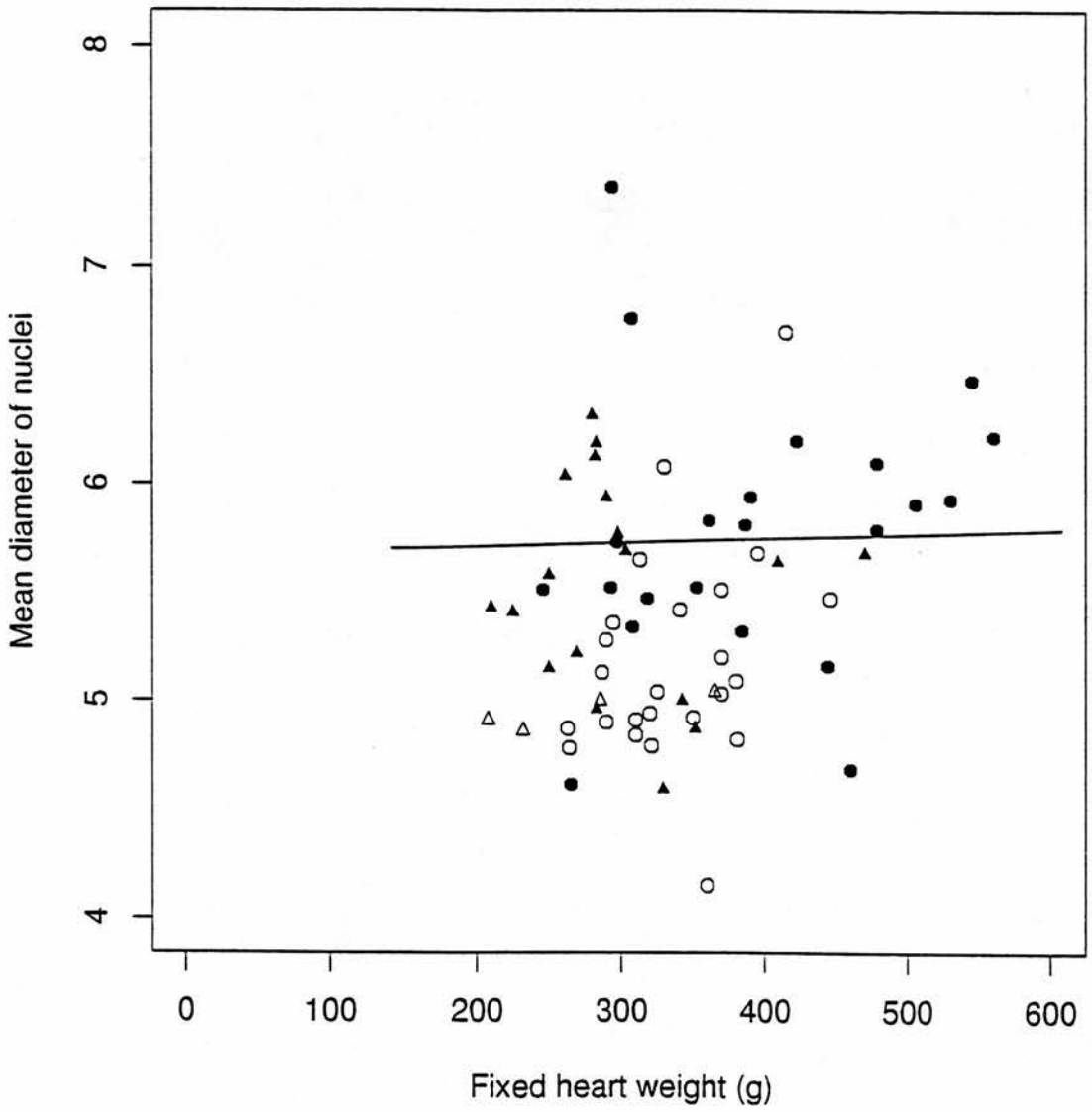

Figure 6.5.6 Mean diameter of nuclei plotted against fixed heart weight.

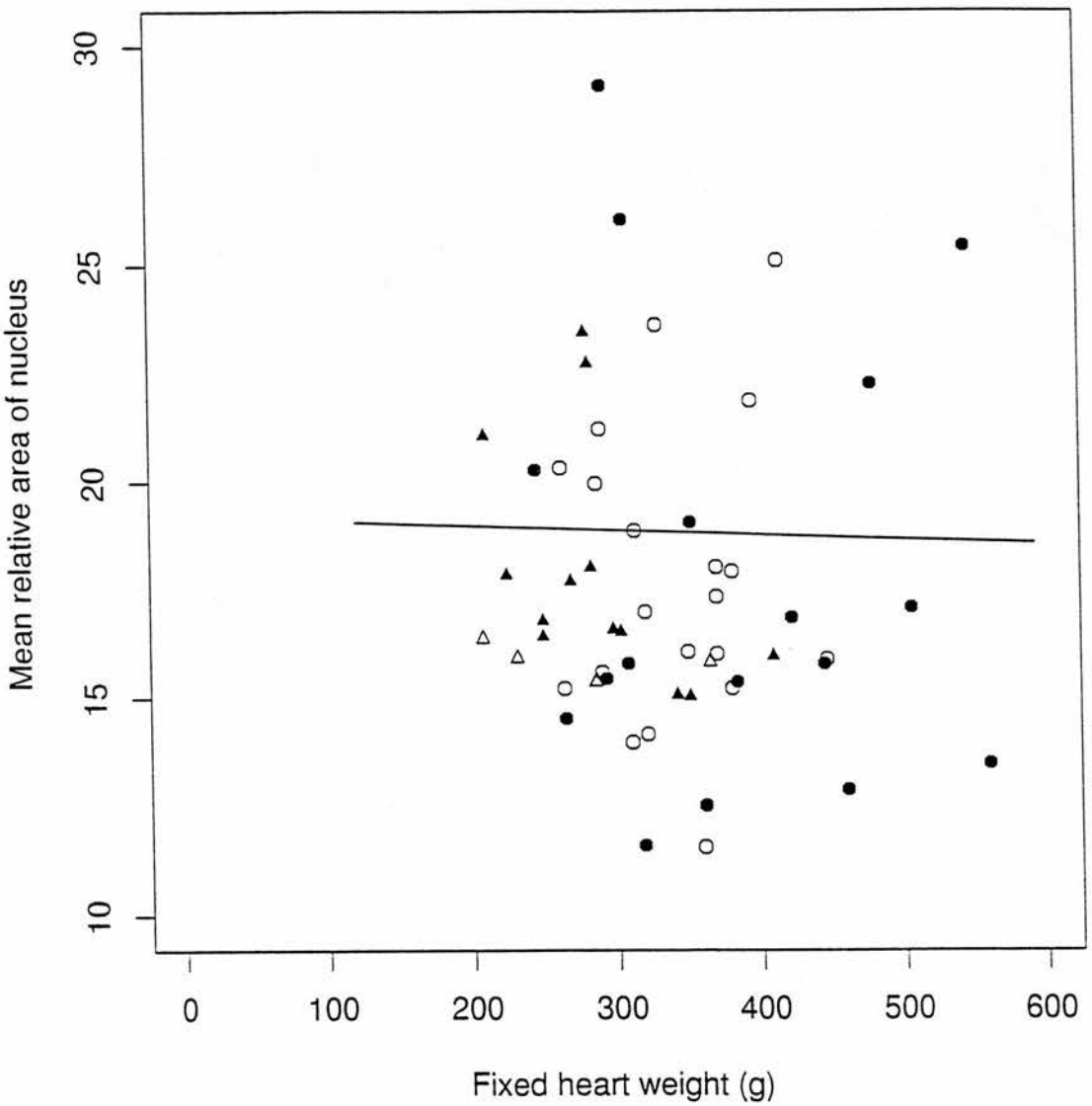

Figure 6.5.7: Mean relative area of nucleus plotted against fixed heart weight.

Table 6.6.1: Comparison of measured variables in different regions of left ventricular walls in controls, with significance test.

Measurement	**D vs C, F**	**E vs C, D, F**
Diameter of muscle	NS	NS
Area muscle	NS	NS
Area connective tissue	NS	NS
% connective tissue	NS	NS
Area nucleus	NS	P<0.05
Diameter nucleus	NS	P<0.05

The comparisons which are referred to in the table above, are between the anterior, lateral and posterior wall of the left ventricle of the heart. There was no statistical significant variation in any of the variables measure that could be shown in between these regions.

A comparison between the septum and the other regions of the left ventricle in these controls showed variations which attained statistical significance in both the area of the fibres occupied by nuclei and the diameters of these nuclei.

6.7 Comparisons of the Right and Left Ventricular Walls in Controls

In order to examine any difference in normal human heart between right ventricle and left ventricle, the results obtained for various parameters from both ventricles in this study were compared with statistical analysis. The results are shown in table 6.7.1.

Table 6.7.1: Comparison of measured variables in right and left ventricles of the heart in controls, with significance test.

Measurement	**B Vs C-F**
Diameter of muscle	P<0.001
Area muscle	P<0.05
Area connective tissue	NS
% connective tissue	NS
Area nucleus	P<0.05
Diameter nucleus	P<0.05

A larger variation was noted when the anterior wall of the right ventricle was compared with the mean results from the left ventricular regions (table 6.7.1). Statistically significant results were as follows: mean diameter of muscle fibres, mean area occupied by muscle fibres and increased mean diameter and area of nuclei in the left ventricle when compared with the right.

The same comparisons which were carried out in the controls were attempted in relation to the alcoholic hearts.

6.8 Comparisons of different Regions of the Left Ventricle in Alcoholics

In order to see if there is any difference in different regions of the left ventricle in alcoholic heart, the results obtained for the measured variables from the different wall of the left ventricle in this study were compared with statistical analysis. The results are shown in table 6.8.1.

Table 6.8.1: Comparison of measured variables in different regions of left ventricular walls in alcoholics, with significance test.

Measurement	**D Vs C, F**	**E Vs C, D, F**
Diameter of muscle	P<0.001	NS
Area of muscle	NS	NS
Area of Connective tissue	NS	P<0.05
% connective tissue	NS	P<0.05
Area of nuclei	NS	P<0.05
Diameter of nuclei	NS	P<0.01

When a comparison was made between the different regions examined in the left ventricle, the significant results were as follows: mean muscle fibre diameters were greater (P<0.001) in lateral wall (region D) than in anterior wall and posterior wall (region C and F). The area and percentage connective tissue were greater (P<0.05) in septum (region E) than in anterior, lateral and posterior wall (region C, D, and F), and mean area and diameter of nuclei was greater (P<0.05 and P<0.01 respectively) in anterior, lateral and posterior wall (region C, D, and F) than in septum (region E) .

These variations are therefore much more pronounced than those identified in the controls.

6.9 Comparison of the Right and Left Ventricle in Alcoholics

Similarly, the comparisons made for the controls between the right and left ventricles were also attempted for the alcoholics to examine any difference between them from the results obtained for different variables. The results are shown in table 6.9.1.

Table 6.9.1: Comparisons of different variables in the right and left ventricles of alcoholic hearts, with significance test.

Measurement	**B Vs C, D, E, F**
Diameters of muscle	P<0.001
Area of muscle	NS
Area of connective tissue	NS
% connective tissue	NS
Area of nuclei	NS
Diameters of nuclei	P<0.05

When the anterior wall (region B) of the right ventricle was compared (see table) to the average of the four regions i.e. anterior, lateral and posterior wall and the septum of the left ventricle (regions C, D, F and E), means were significantly lower for diameters of muscle (P<0.001) and diameters of nuclei (P<0.05), with all other comparisons being non-significant.

6.10 Coefficients of Variation (CVs) for Muscle, Connective Tissue and Nuclear Studies

As described in chapter 3, coefficients of variation, defined as the ratio of standard deviation to the mean, are calculated for the different measurements taken. The mean coefficients of variation are listed in the following tables.

Table 6.10.1. Mean coefficients of variation for muscle fibres measurements. Significance refers to result of two-sample t-test for group difference.

Measurement	Region	Alcoholics	Controls	Significance
Diameter of muscle	B	16	16	NS
Diameter of muscle	C	16	15	P<0.05
Diameter of muscle	D	17	16	NS
Diameter of muscle	E	17	15	NS
Diameter of muscle	F	16	15	NS
Area of muscle	B	11	10	NS
Area of muscle	C	9	9	NS
Area of muscle	D	10	7	P<0.001
Area of muscle	E	9	8	NS
Area of muscle	F	9	8	NS

Table 6.10.2. Mean coefficients of variation for connective tissue measurements. Significance refers to result of two-sample t-test for group difference.

Measurement	Region	Alcoholics	Controls	Significance
Area of connective tissue	B	28	30	NS
Area of connective tissue	C	22	29	P<0.01
Area of connective tissue	D	24	30	P<0.05
Area of connective tissue	E	24	32	P<0.01
Area of connective tissue	F	25	35	P<0.01

Table 6.10.3. Mean coefficients of variation for nucleus measurements. Significance refers to result of two-sample t-test for group difference.

Measurement	Region	Alcoholics	Controls	Significance
Area of nucleus	B	42	35	P<0.01
Area of nucleus	C	42	36	P<0.05
Area of nucleus	D	41	36	P<0.01
Area of nucleus	E	39	36	NS
Area of nucleus	F	40	36	NS
Diameter of nucleus	B	26	23	NS
Diameter of nucleus	C	25	24	NS
Diameter of nucleus	D	25	25	NS
Diameter of nucleus	E	24	24	NS
Diameter of nucleus	F	25	24	NS

Table 6.10.1. to 3 show the means for the CVs of each measurement: the most consistently significant results are for area of nucleus, where it appears that alcoholics not only have greater average areas but also show more proportional variation amongst individual nuclei.

Chapter 7

Results of Blood Vessel Measurements

Introduction

To assess the changes in the microvascular component of the myocardium in alcoholics as compared to controls, comparisons were made of various vascular variables which had been measured.

7.1 Eccentricity of the Measured Blood Vessels

It was patently obvious from microscopical examination of the tissue sections that not all the blood vessels which one wished to measure were neatly sectioned in their transverse plane. The cross sections of blood vessels which could be seen were often oval or elongated in section indicating that they had been cut in a tangential plane. In addition there was a significant probability that fixation could alter the configuration of blood vessels in a non-uniform fashion given that the blood vessels within connective tissue in the interfascicular planes and may react to the chemical fixative in a manner which differs from muscle. A vessel may collapse and from a uniform rounded cylindrical shape be converted to a flattened cylinder with an oval cross-sectional configuration.

The correction formula (discussed in chapter 3) was devised to attempt to correct for these artefacts of eccentricity. To assess whether there was any variation in the manner in which the various vascular coats were affected by postmortem changes and section preparation, it was decided to investigate each coat of the vessel wall separately to identify any changes in configuration. The maximum and minimum diameters of the vascular lumen, the maximum and minimum diameters of the internal elastic lamina (IEL) and external elastic lamina (EEL) as well as the maximum and minimum diameters of the intimal layer (i.e. IEL bound area – Luminal area) and of the medial layer (i.e. EEL bound area – IEL bound area) were compared.

Mean "eccentricities" which were obtained by expressing the minimum diameter as a percentage of the maximum diameter. This figure should equate to 100% for circular vessels and shall result in smaller values for vessels of a very elongated shape, and this is therefore a measure of the degree of collapse. Comparisons were made between the alcoholic and control hearts for the various regional measurements are shown in the following five tables of this section (7.1).

Table 7.1.1 Mean eccentricity of lumen (minimum diameter as percentage of maximum diameter). Significance refers to result of two-sample t-test for group difference.

Measurement	**Region**	**Alcoholics**	**Controls**	**Sig.**
Eccentricity of lumen	B	65	65	NS
Eccentricity of lumen	C	59	60	NS
Eccentricity of lumen	D	59	59	NS
Eccentricity of lumen	E	59	63	P<0.01
Eccentricity of lumen	F	65	62	NS

Legend for table 7.1.1 and subsequent tables: B= anterior wall of right ventricle; C= anterior wall of left ventricle; D= lateral wall of left ventricle; E= inter ventricular septum ; F= posterior wall of left ventricle

Table 7.1.2 Mean eccentricity of IEL (minimum diameter as percentage of maximum diameter). Significance refers to result of two-sample t-test for group difference.

Measurement	Region	Alcoholics	Controls	Sig.
Eccentricity of IEL	B	70	69	NS
Eccentricity of IEL	C	64	65	NS
Eccentricity of IEL	D	64	67	NS
Eccentricity of IEL	E	66	67	NS
Eccentricity of IEL	F	71	67	NS

Table 7.1.3 Mean eccentricity of EEL (minimum diameter as percentage of maximum diameter). Significance refers to result of two-sample t-test for group difference.

Measurement	Region	Alcoholics	Controls	Sig.
Eccentricity of EEL	B	72	73	NS
Eccentricity of EEL	C	68	68	NS
Eccentricity of EEL	D	68	70	NS
Eccentricity of EEL	E	72	71	NS
Eccentricity of EEL	F	72	71	NS

Table 7.1.4 Mean eccentricity of intima (minimum diameter as percentage of maximum diameter). Significance refers to result of two-sample t-test for group difference.

Measurement	**Region**	**Alcoholics**	**Controls**	**Sig.**
Eccentricity of intima	B	94	91	NS
Eccentricity of intima	C	93	91	NS
Eccentricity of intima	D	96	92	NS
Eccentricity of intima	E	99	92	P<0.001
Eccentricity of intima	F	95	93	NS

Table 7.1.5 Mean eccentricity of media (minimum diameter as percentage of maximum diameter). Significance refers to result of two-sample t-test for group difference.

Measurement	**Region**	**Alcoholics**	**Controls**	**Sig.**
Eccentricity of media	B	81	83	NS
Eccentricity of media	C	82	79	NS
Eccentricity of media	D	80	80	NS
Eccentricity of media	E	86	83	NS
Eccentricity of media	F	80	84	NS

The above tables show mean values for eccentricity as compared with the hypothetical ellipses in each group. The figures for the intima were highest, indicating that this feature was closest to circular on average and this **showed least compression**. There were two significant group differences. Eccentricity of lumen in region E is greater in the controls (P<0.01) but eccentricity of intima is greater in the alcoholics compared with controls. (P<0.001).

The differences amongst the regions in the alcoholics were mostly consistent with the findings above, e.g. eccentricity of lumen was greater for B and F than for C, D and E .

7.2 Comparison of Circumferential Length of the Internal Elastic Lamina (IEL) of Blood Vessels

The total circumferential length of IEL and its maximum and minimum diameters were measured. This layer of the vessel wall is probably the least affected variable in postmortem fixation and the slide preparation as has been shown in the studies carried out by Fernie and Lamb (1985), and can therefore be taken, as postulated by them, as a standard variable for the assessment of the size of the blood vessels being measured. By comparing the IEL circumferential length between the vessels in the different regions studied it is possible to state whether or not vessels of a similar size are being compared in the two groups. Furthermore, given the assumption made already regarding the stability of this layer, its measurements could form the basis on which other measurements could referred to and assessed.

In table 7.2.1, a comparison is made between circumferential length of IEL measurements in the different regions of the hearts from the alcoholics and controls.

Table 7.2.1 Mean (sd) of mean circumferential length of IEL measurements. Significance refers to result of two-sample t-test for group difference.

Measurement (μm)	**Region**	**Alcoholics**	**Controls**	**Sig.**
Length of IEL	B	99.70 (17.58)	103.27 (21.99)	NS
Length of IEL	C	108.83 (26.08)	100.38 (26.37)	NS
Length of IEL	D	109.32 (26.33)	94.59 (17.01)	P<0.01
Length of IEL	E	126.22 (35.91)	113.30 (29.74)	NS
Length of IEL	F	112.70 (26.75)	108.19 (23.09)	NS

It is shown that apart from the measurements in the lateral wall (region D) of the left ventricle, the vessels being assessed in the other regions are of comparable size. However, subsequent comparisons were adjusted for length of IEL in order to ensure that vessels of similar size in both alcoholics and controls were compared. This adjustment also gives an increase in statistical power since length of IEL is correlated with most of the measurements.

7.3 Correction of Actual Measurements to Estimate Figures for the Diameter of a Hypothetical Circle

To further assess whether this lack of differences between the blood vessels in the two groups in this study is genuine, the non-circular vascular measurements were corrected arithmetically to their equivalent in terms of the nearest regular circular configuration. Alterations in diameter and length resulted from such conversions from a non-rounded distorted cross section of a vessel in section to one with a more uniform and circular cross-section.

The correction formula used for obtaining the diameter of a hypothetical circle with equivalent circumference of an ellipse from the actual measurements obtained is:

$$(\text{equivalent diameter})^2 = [(\text{Dmax})^2 + (\text{Dmin})^2] / 2$$

Comparison of the altered diameter produced by such mathematical manipulation which have corrected non-rounded blood vessels to more regular transverse cross-section for the various diameters of the vessel wall. Tables in this section (7.3) show figures for the mean diameters of the hypothetical circles with equivalent circumference to the ellipses as calculated from the maximum and minimum diameters, together with tests for differences adjusted for length of IEL.

Table 7.3.1: Mean diameters of hypothetical circles with circumference equivalent to ellipse for luminal measurements. Significance refers to test for group difference adjusted for mean length of IEL.

Measurement (μm)	**Region**	**Alcoholics**	**Controls**	**Sig.**
Diameter of lumen	B	24.28	24.52	P<0.01
Diameter of lumen	C	26.35	23.98	NS
Diameter of lumen	D	26.83	22.97	NS
Diameter of lumen	E	30.97	27.17	NS
Diameter of lumen	F	27.28	26.01	NS

Table 7.3.2: Mean diameters of hypothetical circles with circumference equivalent to ellipse for IEL measurements. Significance refers to test for group difference adjusted for mean length of IEL.

Measurement (μm)	**Region**	**Alcoholics**	**Controls**	**Sig.**
Diameter of IEL	B	29.16	29.78	NS
Diameter of IEL	C	31.67	29.08	NS
Diameter of IEL	D	31.85	27.60	P<0.01
Diameter of IEL	E	36.01	32.80	NS
Diameter of IEL	F	32.20	31.39	NS

Table 7.3.3: Mean diameters of hypothetical circles with circumference equivalent to ellipse for EEL measurements. Significance refers to test for group difference adjusted for mean length of IEL.

Measurement (μm)	**Region**	**Alcoholics**	**Controls**	**Sig.**
Diameter of EEL	B	39.38	40.07	NS
Diameter of EEL	C	41.28	38.69	NS
Diameter of EEL	D	41.52	36.62	NS
Diameter of EEL	E	47.17	43.33	NS
Diameter of EEL	F	42.42	41.04	NS

Table 7.3.4: Mean diameters of hypothetical circles with circumference equivalent to ellipse for intimal measurements. Significance refers to test for group difference adjusted for mean length of IEL.

Measurement (μm)	**Region**	**Alcoholics**	**Controls**	**Sig.**
Diameter of intima	B	2.48	2.67	P<0.01
Diameter of intima	C	2.68	2.60	NS
Diameter of intima	D	2.57	2.57	NS
Diameter of intima	E	2.86	2.75	NS
Diameter of intima	F	2.66	2.74	NS

Table 7.3.5: Mean diameters of hypothetical circles with circumference equivalent to ellipse for medial measurements. Significance refers to test for group difference adjusted for mean length of IEL.

Measurement (μm)	**Region**	**Alcoholics**	**Controls**	**Sig.**
Diameter of media	B	5.11	5.16	NS
Diameter of media	C	4.88	4.82	NS
Diameter of media	D	4.88	4.53	NS
Diameter of media	E	5.61	5.38	NS
Diameter of media	F	4.96	4.86	NS

The tables above indicate that whatever artefactual changes had taken place in the production of the sections for vascular measurements are affecting both the non-alcoholic hearts as well as the alcoholic hearts. It also appears that these artefactual changes are affecting the vessel wall in a more or less uniform fashion.

The significant results are that diameter of lumen and intima are greater in region B in the controls than in the alcoholics ($P<0.01$) but diameter of IEL is greater in region D in the alcoholics compared with the controls ($P<0.01$).

In the tests for differences amongst regions (alcoholics only), there were no significant results for the comparison of C, D and F while both intima and media showed greater equivalent diameters in region E than in C/D/F at $P<0.01$. The lack of significant difference for lumen and EEL is again consistent with the results above, and suggests that alcoholics have similar sizes for these features in C/D and in F but that the degree of compression differs.

7.4 Comparison of Diameters of the Area Bounded by the Internal Elastic Lamina (IEL) of Blood Vessels

The internal diameter of a blood vessel is considered to be represented by the IEL for this study, as discussed in chapter 3. The circumferential length of IEL was selected using the grey scale of the IBAS machine in order to obtain its total circumferential length and maximum and minimum diameters. Comparisons of the diameters were made between the two groups. The results are listed in table 7.4.1.

Table 7.4.1: Mean (sd) of mean diameters of IEL measurements. Significance refers to two-sample t-test for group difference adjusted for mean length of IEL.

Measurement (μm)	**Region**	**Alcoholics**	**Controls**	**Sig.**
Diameter (max) of IEL	B	33.87 (5.92)	34.65 (7.28)	NS
Diameter (max) of IEL	C	37.85 (9.56)	34.50 (8.00)	NS
Diameter (max) of IEL	D	37.96 (9.38)	32.43 (5.84)	NS
Diameter (max) of IEL	E	42.71 (11.98)	38.54 (9.36)	NS
Diameter (max) of IEL	F	37.05 (7.45)	36.96 (7.90)	NS
Diameter (min) of IEL	B	23.48 (4.18)	23.90 (4.51)	NS
Diameter (min) of IEL	C	23.82 (4.51)	22.32 (6.22)	NS
Diameter (min) of IEL	D	24.14 (4.55)	21.67 (3.83)	NS
Diameter (min) of IEL	E	27.67 (6.86)	25.77 (6.15)	NS
Diameter (min) of IEL	F	26.29 (6.65)	24.48 (4.57)	NS

After adjustment for the length of IEL no statistical significant difference was identified in relation to the maximum and minimum diameters of IEL between the two groups.

7.5 Comparison of the Area and Diameters of the Lumen of Blood Vessels

To assess any changes in the lumen of alcoholic vessels compared to controls the total area of the lumen and its maximum and minimum diameters were measured. The results are listed in table 7.5.1 and 7.5.2.

Table 7.5.1. Mean (sd) of mean luminal area measurements. Significance refers to two-sample t-test for group difference adjusted for mean length of IEL.

Measurement (μm^2)	**Region**	**Alcoholics**	**Controls**	**Sig.**
Area of lumen	B	468.94 (213.55)	512.03 (327.73)	NS
Area of lumen	C	531.41 (338.47)	478.23 (469.34)	NS
Area of lumen	D	525.56 (302.15)	407.89 (289.65)	NS
Area of lumen	E	744.69 (566.24)	610.41 (393.91)	NS
Area of lumen	F	625.95 (433.66)	532.15 (266.85)	NS

In table 7.5.1 alcoholics showed no statistical significant difference in the measurement of area of the lumen of blood vessel as compared to controls.

Table 7.5.2. Mean (sd) of mean luminal diameters measurement. Significance refers to two-sample t-test for group difference adjusted for mean length of IEL.

Measurement (μm)	**Region**	**Alcoholics**	**Controls**	**Sig.**
Diameter (max) of lumen	B	28.74 (5.67)	29.07 (7.06)	P<0.05
Diameter (max) of lumen	C	32.17 (9.31)	29.03 (8.01)	NS
Diameter (max) of lumen	D	32.71 (9.05)	27.89 (6.51)	NS
Diameter (max) of lumen	E	37.80 (12.62)	32.45 (8.88)	NS
Diameter (max) of lumen	F	32.23 (8.39)	31.27 (7.61)	NS
Diameter (min) of lumen	B	18.71 (4.03)	18.84 (4.42)	NS
Diameter (min) of lumen	C	18.65 (4.51)	17.43 (6.20)	NS
Diameter (min) of lumen	D	19.12 (4.25)	16.26 (4.80)	NS
Diameter (min) of lumen	E	21.99 (6.36)	20.50 (5.59)	P<0.05
Diameter (min) of lumen	F	21.11 (6.50)	19.25 (4.46)	NS

The only statistical significant difference was identified in the right ventricle and the septum of the left ventricle, with all other areas non-significant.

7.6 Comparison of Measured Area and Thickness of the Intima of Blood Vessels

To determine whether there is thickening of the intimal coat of the intra-myocardial arterioles in alcoholic hearts, specific morphometric measurements were made for IEL length and diameters of the area bounded by IEL and the area and diameters of the lumen. By simple calculation, figures were obtained for the area occupied by the intima and the maximal and minimal thickness of the intimal coat.

The mean values for these measurements i.e. the total area of intima and its thickness (although the short-hand term "diameter" is used throughout) were obtained by calculating the mean value of all the measurements taken in the five

regions of the heart which were examined and were compared with identical measurements in the controls (see tables 7.6.1 and 7.6.2).

Table 7.6.1. Mean (sd) of mean intimal area measurements. Significance refers to two-sample t-test for group difference adjusted for mean length of IEL.

Measurement (μm^2)	**Region**	**Alcoholics**	**Controls**	**Sig.**
Area of intima	B	219.68 (62.04)	239.83 (71.27)	NS
Area of intima	C	262.20 (95.70)	226.73 (72.95)	NS
Area of intima	D	260.49 (127.72)	215.43 (63.25)	NS
Area of intima	E	336.82 (146.73)	287.73 (128.14)	NS
Area of intima	F	273.00 (97.71)	260.80 (83.10)	NS

These comparisons show that there is no statistically significant difference between two groups as far as the area of intima is concerned, thus indicating that no intimal thickening of blood vessels is found in either the left ventricle or the right ventricle in alcoholics as compared to non-alcoholic controls.

Table 7.6.2. Mean (sd) of mean intimal thickness (diameter) measurements. Significance refers to two-sample t-test for group difference adjusted for mean length of IEL.

Measurement (μm)	**Region**	**Alcoholics**	**Controls**	**Sig.**
Diameter (max) of intima	B	2.57 (0.25)	2.79 (0.31)	P<0.01
Diameter (max) of intima	C	2.78 (0.35)	2.74 (0.41)	NS
Diameter (max) of intima	D	2.63 (0.31)	2.67 (0.34)	NS
Diameter (max) of intima	E	2.87 (0.40)	2.86 (0.46)	NS
Diameter (max) of intima	F	2.72 (0.39)	2.85 (0.46)	NS
Diameter (min) of intima	B	2.40(0.21)	2.53 (0.21)	P<0.05
Diameter (min) of intima	C	2.58 (0.41)	2.45 (0.19)	NS
Diameter (min) of intima	D	2.51 (0.34)	2.45 (0.30)	NS
Diameter (min) of intima	E	2.84 (0.44)	2.63 (0.40)	NS
Diameter (min) of intima	F	2.59 (0.47)	2.62 (0.30)	NS

As a further indication of any intimal thickening which may be present, a comparison was made between the diameters of the intima which had been adjusted for the configuration of the blood vessels within the tissue sections. With respect to the diameters the only figure to attain statistical significance was that for the diameter of intima in the right ventricle and this was the case for both maximal and minimal diameters. However, on detailed histological examination of these vessels there was no obvious difference in their appearances or in the deposition of intimal or subendocardial substances in the right ventricular vessels as compared with the vessels in the left ventricle.

In figure 7.6.1, a plot of mean maximum diameter of intima, region B, plotted against mean length of IEL shows that the correlation with length of IEL is less strong in this instance after adjustment for length of IEL.

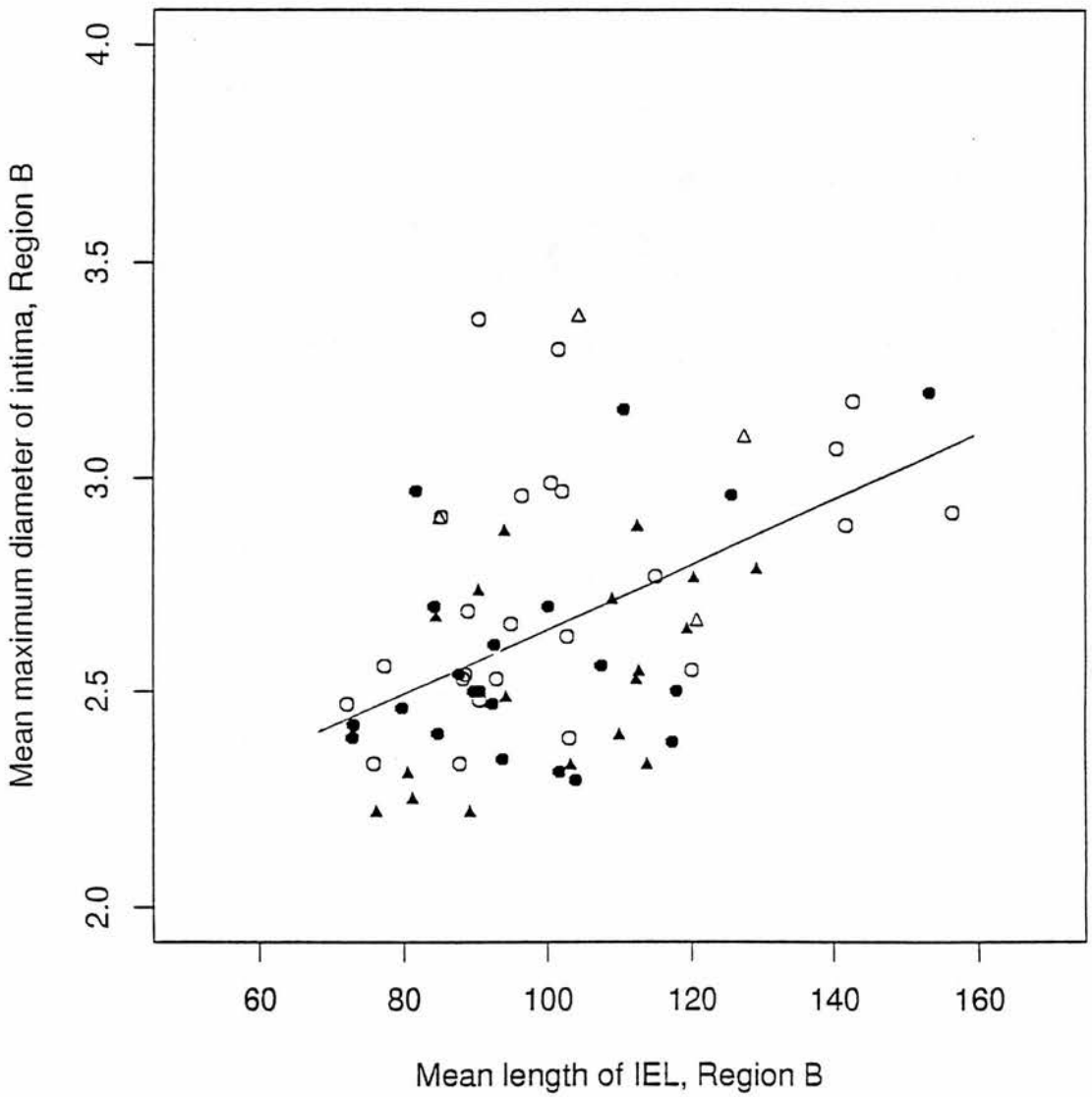

Figure 7.6.1 A plot of mean maximum diameter of intima (region B) plotted against mean length of IEL.

7.7 Comparison of Measurements of the External Elastic Lamina (EEL) of Blood Vessels

As already discussed in chapter 3, the external diameter of a blood vessel is considered to be represented by the EEL for this study. The external elastic lamina was traced to obtain its total circumferential length and maximum and minimum diameters. Comparisons were made between the two groups and only the results for the diameters are listed in table 7.7.1, as the EEL circumferential length is known not to be as constant variable as the IEL.

Table 7.7.1 Mean (sd) of mean diameters of EEL measurements. Significance refers to two-sample t-test for group difference adjusted for mean length of IEL.

Measurement (μm)	**Region**	**Alcoholics**	**Controls**	**Sig.**
Diameter (max) of EEL	B	45.16 (7.75)	45.83 (9.89)	NS
Diameter (max) of EEL	C	48.40 (10.97)	45.29 (10.35)	NS
Diameter (max) of EEL	D	48.69 (11.03)	42.39 (6.84)	NS
Diameter (max) of EEL	E	54.30 (14.02)	49.92 (12.92)	NS
Diameter (max) of EEL	F	48.69 (9.50)	47.48 (9.47)	NS
Diameter (min) of EEL	B	32.54 (5.75)	33.30 (7.14)	NS
Diameter (min) of EEL	C	32.53 (5.90)	30.63 (8.01)	NS
Diameter (min) of EEL	D	32.73 (6.00)	29.68 (5.27)	NS
Diameter (min) of EEL	E	38.49 (9.32)	35.46 (9.28)	NS
Diameter (min) of EEL	F	34.99 (7.39)	33.31 (6.02)	NS

Alcoholics show no significant difference in their maximum and minimum diameter of EEL as compared to controls.

Figure. 7.7.1 shows a typical plot of the data (mean maximum diameter of EEL, region B), illustrating the strong dependence on length of IEL and the lack of difference between groups adjusted for this.

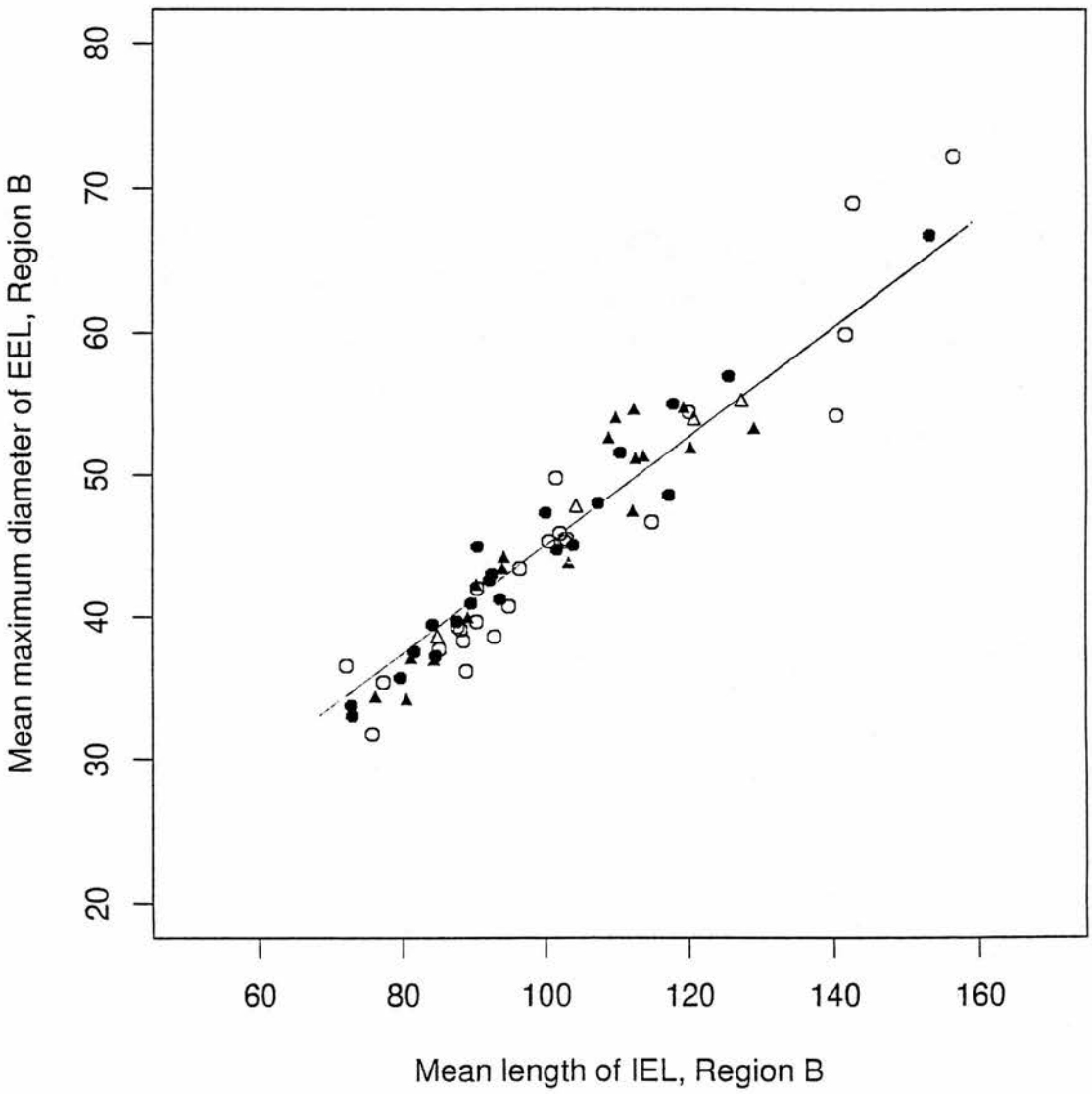

Figure. 7.7.1 Maximum diameter of EEL (region B) plotted against mean length of IEL.

7.8 Comparison of Measurements of the Media

Another possible variation between the blood vessels in alcoholic hearts and the controls is thickening of the medial layer. To assess whether such a change is indeed present, the thickness of the media was assessed by comparison of the diameter of the media (calculated from maximum and minimum diameters of EEL–IEL maximum and minimum diameters) and the area of the media (calculated from area EEL – area IEL). The results are listed in table 7.8.1.

Table 7.8.1 Mean (sd) of mean medial area measurements. Significance refers to two-sample t-test for group difference adjusted for mean length of IEL.

Measurement (μm^2)	**Region**	**Alcoholics**	**Controls**	**Sig.**
Area of media	B	638.59 (280.43)	713.01 (474.81)	NS
Area of media	C	643.57 (380.66)	614.87 (442.38)	P<0.05
Area of media	D	639.01 (333.01)	558.38 (296.23)	P<0.05
Area of media	E	902.66 (487.42)	831.83 (608.10)	NS
Area of media	F	701.17 (295.71)	661.29 (337.15)	NS

In the comparisons of the measurement of area of the media between the two groups the only statistically significant difference shown in the anterior and lateral wall of the left ventricle where alcoholics showed increased values as compared with controls.

Table 7.8.2 Mean (sd) of mean medial thickness (diameter) measurements. Significance refers to two-sample t-test for group difference adjusted for mean length of IEL.

Measurement (μm)	**Region**	**Alcoholics**	**Controls**	**Sig.**
Diameter (max) of media	B	5.62 (1.20)	5.59 (1.71)	NS
Diameter (max) of media	C	5.33 (1.17)	5.39 (1.61)	NS
Diameter (max) of media	D	5.36 (1.23)	4.98 (0.97)	NS
Diameter (max) of media	E	6.00 (1.37)	5.85 (2.17)	NS
Diameter (max) of media	F	5.48 (1.17)	5.26 (1.36)	NS
Diameter (min) of media	B	4.54 (1.03)	4.68 (1.66)	NS
Diameter (min) of media	C	4.36 (1.17)	4.16 (1.07)	NS
Diameter (min) of media	D	4.30 (0.96)	4.00 (1.04)	NS
Diameter (min) of media	E	5.16 (1.21)	4.85 (1.76)	NS
Diameter (min) of media	F	4.35 (0.85)	4.41 (1.22)	NS

As far as the diameter of media is concerned there was no significant difference for the calculated figures obtained for the alcoholics as compared to controls.

Overall in sections 7.4 to 7.8 there are 65 significance tests carried out, of which 5 are significant at the 5% level and one at the 1% level: this is approximately what would be expected by chance alone, and suggests that there are no consistent differences between alcoholics and controls in the dimensions of their blood vessels after standardisation for length of IEL.

7.9 Coefficients of Variation for Blood Vessel Measurements

Coefficient of variation is defined as the ratio of the standard deviation to the mean, expressed as a percentage. This is a measure of proportional variation, and is more appropriate than the standard deviation itself when individual subjects show a wide range of mean values, since it adjusts each individual standard deviation (sd) for difference in mean.

Table 7.9.1: Mean coefficients of variation for the measurements of the area of lumen, intima and media of blood vessels. Significance refers to result of two-sample t-test.

Measurement	**Region**	**Alcoholics**	**Controls**	**Sig.**
Area of lumen	B	86	94	NS
Area of lumen	C	87	86	NS
Area of lumen	D	75	87	NS
Area of lumen	E	90	94	NS
Area of lumen	F	95	91	NS
Area of intima	B	56	61	NS
Area of intima	C	59	54	NS
Area of intima	D	51	57	NS
Area of intima	E	62	61	NS
Area of intima	F	60	60	NS
Area of media	B	79	84	NS
Area of media	C	95	76	NS
Area of media	D	71	89	NS
Area of media	E	83	88	NS
Area of media	F	86	79	NS

Table 7.9.2: Mean coefficients of variation for the length of IEL measurement. Significance refers to result of two-sample t-test.

Measurement	Region	Alcoholics	Controls	Sig.
Length of IEL	B	37	43	NS
Length of IEL	C	38	38	NS
Length of IEL	D	35	38	NS
Length of IEL	E	43	43	NS
Length of IEL	F	42	42	NS

Table 7.9.3: Mean coefficients of variation for the measurements of the luminal diameters. Significance refers to result of two-sample t-test.

Measurement	Region	Alcoholics	Controls	Sig.
Diameter (max) of lumen	B	43	48	NS
Diameter (max) of lumen	C	42	44	NS
Diameter (max) of lumen	D	40	44	NS
Diameter (max) of lumen	E	46	46	NS
Diameter (max) of lumen	F	46	47	NS
Diameter (min) of lumen	B	42	45	NS
Diameter (min) of lumen	C	44	41	NS
Diameter (min) of lumen	D	38	54	NS
Diameter (min) of lumen	E	45	47	NS
Diameter (min) of lumen	F	48	44	NS

Table 7.9.4: Mean coefficients of variation for the measurements of the IEL diameters. Significance refers to result of two-sample t-test.

Measurement	Region	Alcoholics	Controls	Sig.
Diameter (max) of IEL	B	38	42	NS
Diameter (max) of IEL	C	38	38	NS
Diameter (max) of IEL	D	36	38	NS
Diameter (max) of IEL	E	41	40	NS
Diameter (max) of IEL	F	42	42	NS
Diameter (min) of IEL	B	35	38	NS
Diameter (min) of IEL	C	37	34	NS
Diameter (min) of IEL	D	32	35	NS
Diameter (min) of IEL	E	38	41	NS
Diameter (min) of IEL	F	40	38	NS

Table 7.9.5 Mean coefficients of variation for the measurements of the EEL diameters. Significance refers to result of two-sample t-test.

Measurement	Region	Alcoholics	Controls	Sig.
Diameter (max) of EEL	B	36	40	NS
Diameter (max) of EEL	C	36	37	NS
Diameter (max) of EEL	D	35	39	NS
Diameter (max) of EEL	E	40	39	NS
Diameter (max) of EEL	F	39	40	NS
Diameter (min) of EEL	B	36	38	NS
Diameter (min) of EEL	C	36	33	NS
Diameter (min) of EEL	D	31	36	NS
Diameter (min) of EEL	E	37	40	NS
Diameter (min) of EEL	F	40	36	NS

Table 7.9.6 Mean coefficients of variation for the measurements of the intimal diameters. Significance refers to result of two-sample t-test.

Measurement	Region	Alcoholics	Controls	Sig.
Diameter (max) of intima	B	21	23	NS
Diameter (max) of intima	C	24	22	P<0.05
Diameter (max) of intima	D	24	20	P<0.01
Diameter (max) of intima	E	26	23	NS
Diameter (max) of intima	F	21	25	NS
Diameter (min) of intima	B	22	22	NS
Diameter (min) of intima	C	23	19	NS
Diameter (min) of intima	D	22	20	NS
Diameter (min) of intima	E	24	24	NS
Diameter (min) of intima	F	22	20	NS

Table 7.9.7 Mean coefficients of variation for the measurements of the diameters of media. Significance refers to result of two-sample t-test.

Measurement	Region	Alcoholics	Controls	Sig.
Diameter (max) of media	B	42	44	NS
Diameter (max) of media	C	40	41	NS
Diameter (max) of media	D	40	45	NS
Diameter (max) of media	E	42	45	NS
Diameter (max) of media	F	41	38	NS
Diameter (min) of media	B	46	47	NS
Diameter (min) of media	C	43	40	NS
Diameter (min) of media	D	40	49	P<0.05
Diameter (min) of media	E	47	48	NS
Diameter (min) of media	F	47	46	NS

The above tables show that there are few significant differences in the coefficients of variation between the two groups in this study.

Chapter 8

General Discussion

Introduction

The results obtained in the previous chapters are examined here in the context of the original objectives of the project. It is necessary to highlight the applications or limitations of the observations and measurements made and the methods of analysis used throughout this study. Furthermore, areas of potentially rewarding future work arising out of this research are suggested.

8.1 Objectives of the Project

The findings of this study have provided answers to the questions which were being investigated. The hypothesis being tested was that if alcohol is abused over a number of years it induces morphological abnormalities in the myocardium, long before features of a dilated cardiomyopathy can be observed either at autopsy or by clinical diagnosis or by specialised investigations. The second objective was to quantify such effects of ethanol on the myocardial components i.e. myocytes, connective tissue and myocardial arterioles, and to do this by morphometric methods which, being largely free of observer bias, would provide more accurate and reliable measurements.

8.2 Series of Population Studied

8.2.1 Choice of Cases

All the cases studied presented as a sudden unexpected death which required an investigation of a medico-legal type. Part of this investigation was the obtaining

of detailed medical and social history of the deceased and all the cases chosen had a history of many years of excessive alcohol consumption. Information as to the exact amount of alcohol consumed and the exact type of alcoholic beverages used were not always available and indeed such information is very often erroneous. As long as the history had been obtained from reliable sources such as relatives and the general practitioners of the deceased, and this history had been verified by the investigating police officer, the diagnosis of chronic alcoholism was accepted.

The second aspect of selection of these cases related to the findings at an extensive autopsy carried out in all instances by consultant forensic pathologists in accordance with a very detailed protocol. These autopsies also involved the collection of specimens for histology and toxicological analysis. Hearts were only chosen in cases where the death of the patient could be attributed to a condition which was not of cardiac type and when there was no indication that cardiac abnormality was even an ancillary or associated cause of death.

Each heart was very carefully dissected and it was established that there was no valvular abnormality and significant coronary arterial degenerative disease. Cases in which potentially cardiotoxic agents could have been acting on the heart and had been taken prior to death, were also excluded. The intention was to choose hearts which had been exposed over a period of several years to alcohol and to no other potential cardiotoxic agents.

8.2.2 Choice of Controls

In the choice of controls the criteria which were adopted were age and sex matching as much as possible from a population of persons dying suddenly, unexpectedly, and often violently from an acute event, provided that the detailed past medical history obtained in an identical fashion to that of alcoholic patients did not indicate any abuse of alcohol whatsoever or any significant cardiac complaint.

These control hearts were dissected, fixed and examined in a fashion entirely identical to that of the alcoholic hearts, and subjected to similar morphological measurements.

The controls therefore emanated from an identical population reservoir and apart from the absence of the history of chronic alcohol abuse, these deceased persons had lived and died in the same geographical area.

8.2.3 Effects of Fixation

All the heart specimens collected for this study were fixed in formalin and it was found that fixed heart weights were significantly lower than fresh heart weights in both groups ($P<0.001$). Prolonged fixation of organs in 10% neutral buffered formalin for periods over one week is known to cause some secondary shrinkage in the weight of the organ concerned and the heart is no exception to this (Bahr et al, 1957; Lamb, 1973; Ludwig, 1979; Scholz et al, 1988). Scholz et al (1988) stated that formalin fixation generally produces less than 6% change in heart weights. This phenomenon is not accepted by all researchers (e.g. Hangartner et al, 1985) and alterations in cardiac weights are probably directly related to the actual fixative solution used and the specific and idiosyncratic environmental factors in individual laboratories.

In this series when fresh heart weights were compared to fixed heart weights there was a reduction (mean 6%) in the total heart weight in the controls and a diminution (mean 5%) of total heart weight in alcoholics. There was, therefore, no significant difference between the two groups when fixed heart weights were expressed as a percentage of the fresh heart weights. Therefore, the two groups were comparable and not significantly affected by postmortem fixation.

8.2.4 The "Normality" of the Controls

It was important to ensure that the control population used in this study matched in basic characteristics to other normal populations in which cardiac characteristics had been documented. The three main series which had been published in this respect are Lamb (1973), Hangartner et al (1985), and Kitzman et al (1988).

The controls in this series matched in terms of the figures for total heart weights with those of Lamb and Kitzman et al. There was however, some variation from the weights given in Hangartner et al series from London. It is however, noted by the latter authors that their heart specimens had been kept in formalin for periods *"up to 3 months"* and they did not observe any significant shrinkage in heart weights. This is as such is unusual and perhaps casts some shadow on the figures produced in this series.

The controls chosen in this study therefore appear to match previously published data for a 'normal population'. The age matched choice was deliberate so as to ensure that any minor coronary arterial degenerative changes which are to be expected in a Scottish population after the age of 40 particularly in male, were taken into consideration.

The controls in this series when compared to the alcoholics showed no significant difference in body heights and body weights in both sexes. The body mass index, was also used for further comparison and also showed no significant variation.

There were significantly fewer females among the controls than in the alcoholics and this raises the possibility that sex may be a confounding factor. However, the majority of the heart weight analyses were adjusted for sex through standardisation, and there were no significant sex differences after adjustment for body weight in the measurements that were not standardised.

In the analysis of connective tissue and myocyte data there were no significant differences between the sexes for any of the measurements in either group, and so on statistical advice it was not felt necessary to adjust for sex in the analysis of differences between the groups.

8.3 Assessment of Heart Weight

8.3.1 Total Fixed Heart Weight

Heart weight is known to be related to body size, and may also to some extent be predicted on the basis of body height, body weight, and total body surface

area (Kitzman et al 1988). Total heart weight in normal subjects is also known to be increased with age and physical activity (Lamb, 1973) and there is also evidence that ventricular mass is elevated in certain athletes (Rost, 1982; Oakley, 1984). Therefore, studies in which heart weight is said to be increased should relate this finding to overall body measurements.

When matched for age and body size normal heart weight is greater in men than in women, and was reported to be 0.45% of body weight in men and 0.40% of body weight in women (Ludwig, 1979). A significant difference between the average heart weights for males and females was also found by Dadgar et al (1979), who showed that the normal heart weight is greater in males than in females. This study found 0.51% of body weight in males and 0.42% of body weight in females in control hearts, values which are essentially comparable to Ludwig's (1979) findings.

Results of this study indicates that body weight is a better univariate predictor of normal heart weight than is body length and body mass index, and is consistent with the findings of Hayes and Lovell (1966); Dadgar et al (1979), Hangartner et al (1985) and Kitzman et al (1988).

In contrast to the well established relationship between body size and heart weight (Smith, 1928; Zeek, 1942; Prothero, 1979) that between age and heart weight is more controversial. Some investigators have reported that heart weight increases progressively with advancing age (Howell, 1978 & 1981; Hodkinson, 1979) and others have maintained that it decreases in the 9th and 10th decades of life (Waller et al, 1984). Dadgar et al (1979) observed that up to the age of 30 years there was a gradual rise of heart weight and thereafter no correlation with chronological age was observed. In this study data showed that there was no strong trend with age in heart weight adjusted for body weight.

In the internal comparison of the two groups, alcoholics showed significantly increased heart weight adjusted for body weight than the controls in this study. No gender difference was identifiable. The causes for such difference in total heart weight were explained further by looking at individual ventricular measurements and the contribution to heart weight by degree of adiposity.

8.3.2 Fat-Free Left and Right Ventricular Weights

When fat-free muscular portion of the ventricles were separated, a difference was noted when the alcoholic hearts were compared to controls. The fat-free right ventricular weights were significantly higher in alcoholics and although the fat-free left ventricular weights were also increased this did not attain statistical significance. This situation was assessed in a different way by studying the ratio of the left ventricular weights to the right ventricular weights.

Fat-free left ventricular weights in this study are comparable to those published by Fulton et al (1952) and Lamb (1973); all the left ventricular weights in the controls were less than the base line figure for normal left ventricular weight of >225 gm indicated by these authors. They are also comparable to Hangartner et al (1985).

Fat-free right ventricular weights in the present study are not comparable to Hangartner et al (1985), but the right ventricular weights without fat in this study are comparable to Lamb (1973) and were also broadly similar to those of Fulton et al (1952) and McPhie (1957). This apparent discrepancy with Hangartner et al (1985) could be explained by the observation of Fulton et al (1952), who reported that the right ventricle was above the upper limit of normal in about one-third of the cases although there was no independent cause of right ventricular hypertrophy in their study population and suggested that a right ventricular hypertrophy is considered to be present when the free wall of the right ventricle weighs 80 gm or more and a total ventricular weight of over 250 gm or more may be taken as evidence of cardiac hypertrophy.

These data were also subjected to a different analysis by studying the ratio of the left ventricular weight to the right ventricular weight.

8.3.3 Ventricular Weight Ratio

Lamb (1973) suggested the use of the ratio weight of left ventricle with septum to right ventricle (normal 2:1) is the most useful method of identifying mild or moderate degrees of isolated ventricular hypertrophy, even if the actual weights

are within the normal range. Fulton et al (1952) suggested a normal ventricular-weights ratio of 2.3:1 to 3.3:1, and similar values were described by Jones (1953) and by Bove et al (1966). The ratio of the ventricular weights in the controls of this study are comparable to those of Lamb (1973) and were also broadly in agreement with those of Fulton et al (1952), who commented that as the ratio may in itself be misleading, when there is hypertrophy of both ventricles giving a normal ratio, it is essential to consider the actual weights in coming to a conclusion about the presence or degree of hypertrophy.

8.3.4 Contribution of Fat Component of the Heart

Combined fat weight as a percentage of total heart weight was significantly higher ($P<0.05$) in the alcoholics than in the controls.

As this study was concentrated on the ventricular portion of the heart, the contribution which fat may have played was assessed only in the ventricular weights and this was done by comparing weights of ventricles before and after the fat on them had been trimmed. When such comparisons were made it was shown that in alcoholics the weight of the total ventricular fat exceeded that found in controls and this attains statistical significance after adjusting for body weight.

After separation of the ventricles and weighing the fat separately showed that right ventricular wall had a larger weight of fat in it as compared to controls and this also achieves statistical significance. Although the left ventricle also tended to show increase contribution of fat to its weight this did not reach statistical significance. Adiposity appears to be increased in the ventricles of the alcoholic hearts particularly in the right ventricle.

8.4 Morphometric Histological Findings

8.4.1 Techniques Used

The morphometric equipment used for all the measurements reported has been validated in its uses in numerous projects and shown to produce reliable and reproducible measurements. No specific testing, other than reproducibility of measurements, of the programs and equipment used was therefore entered into. Reliable and expert scientific advice and assistance was available throughout the course of this study.

It was ensured before actually starting on any of the cases referred to in this study, that I was trained by qualified technical staff and that familiarised myself thoroughly with the equipment, its limitations and the techniques of measurements. Initially the first few slides were measured under direct and constant supervision of technical and medical staffs who have specialised in the use of this equipment. All the measurements were carried out throughout on controls and alcoholic cases, at random.

8.4.2 Proportional Composition of Myocardium

Overall morphological studies have shown that in the alcoholic hearts muscle fibres occupy a relatively smaller proportion of the tissue section in the left ventricle. This attained statistical significance at the 5% level as compared to the controls, but the proportion of muscle fibre area did not differ significantly in the right ventricle and conversely there was a higher proportion of inter-myocytic connective tissue ($P<0.001$) in the alcoholic hearts. This was confirmed in all the myocardial regions which were selected for the study and this is in accord with studies carried out by others using much more subjective methods of assessment (Brigden and Robinson, 1964; Massumi et al, 1965; Alexander, 1966; Tobin, Jr. et al, 1967; Burch and DePasquale, 1969; Schenk and Cohen, 1970; Brigden, 1972; Hognestad and Teisberg, 1973; Demakis et al, 1974; Edmondson, 1980; Ferris et al, 1981; Teragaki et al, 1993).

Brigden and Robinson (1964) performed autopsies in nine cases and described patchy fibrosis within the myocardium, hypertrophy of the myocardium and focal myocardial degeneration in all chamber of the heart but carried out no morphometric studies. Massumi et al (1965) described myocardial fibre hypertrophy and fibrosis in the alcoholic patients. Alexander (1966) in examination of 100 cases which were randomly selected showed patchy fibrosis particularly noticeable in the subendocardial area. Tobin et al (1967) reported left, and frequently right, ventricular hypertrophy and diffuse interstitial fibrosis. Burch and DePasquale (1969) observed hypertrophied muscle fibres adjacent to atrophic or degenerating muscle fibres and diffuse areas of fibrosis in alcoholics but no specific measurements were made in these studies.

Schenk and Cohen (1970) described the presence of diffuse interstitial fibrosis in 87 of the 97 cases studied and indicated that distribution was non-specific and unlikely to be related to vascular occlusion. Brigden (1972) observed myocardial hypertrophy with great variation in fibre size and patchy myocardial fibrosis. Hognestad and Teisberg (1973) in the investigation of 35 alcoholic hearts observed myocardial hypertrophy, subendocardial, interstitial and perivascular fibrosis. Demakis et al (1974) observed varying degrees of myocyte hypertrophy and fibrosis in seven chronic alcoholic patients but no morphometric studies were carried out in these investigations.

Edmondson (1980) reported on interstitial fibrosis with *"small scars"* that replace muscle fibres. Ferris et al (1981) reported that increased interstitial fibrosis was a common finding in their investigation of alcoholic patients but no measurements were made in these studies.

Teragaki et al (1993) in 4 μm thick sections stained with H&E and EVG evaluated the fibrous component of hearts of alcohol drinkers by a point-counting method applied at a magnification of x400, with an 11x11 points square grid being used and counts made in randomly chosen areas. These authors noticed the percentage fibrosis ranging from 8.9 to 28.0 (mean 14.9 ± 1.2)% in the patients studied but indicated that the degree of fibrosis present did not directly relate to the length of history of alcohol consumption. By contrast, in this study the data for connective tissue area measurement was 9.62% ± 2.72% (mean ± sd). This discrepancy could be explained in that the cases chosen by Teragaki et al, unlike

those in this study, had full-blown alcohol-induced congestive cardiac failure rather than pre-morbid changes, differences in the technical measurement system employed and the difference in the population sample selected i.e. Japanese versus Caucasians. Smaller amounts of ethanol may cause abnormalities including alcoholic cardiomyopathy in Japanese as compared with Caucasians (Harada et al, 1980).

8.4.3 Myocardial Morphometry

It was shown that the heart muscle cells were hypertrophied in both ventricles in the alcoholic hearts as compared to controls. This hypertrophy could be quantitated and indeed corresponding other measurements of the nuclei of the myocardial fibres were also enlarged.

The diameters of myocytes within the myocardium have been recorded by simple measurement technique in various conditions affecting the heart such as atrophy (Karsner et al, 1925), hypertension (Ashley, 1945; Fuster et al, 1977), cor-pulmonale (Ishikawa et al, 1972) both in humans and in primates (Truex, 1972).

In the study of Fuster et al (1977), hearts from patients with mitral incompetence and with hypertension were subjected to a quantitative study to assess left ventricular myocardial fibre hypertrophy and interstitial tissue content. This was done by obtaining photographs of stained histological preparations and measuring the shortest diameter of myocardial fibres at the level of the nucleus. It was shown that the diameters of myocardial fibres, as expected were increased when chronic volume overload was present. The myocardial fibre diameter for normal control hearts was 5.8 ± 0.54 μm (mean ± sd) and the proportion of "interstitial tissue" to myocardial fibre space was 32.2%. The latter is artificial to some extent as no efforts were made to remove the volume of empty space when this ratio was produced. The hypertrophied fibres gave a diameter of 7.1 ± 0.80 μm (mean ± sd) and in hypertensive hearts the amount of connective tissue was not found to be significantly increased. However, in chronic mitral incompetence an increase in connective tissue, up to 50% was identified.

Hoshino et al (1983) addressed their study to an identification of regional variation in myocardial fibre diameter throughout the ventricular wall. The

underlying premise for this study was that intra-cardiac pressure and stress were unevenly distributed throughout heart and should therefore be proportionally represented differently in different regions. The hearts were fixed in formalin, serially sliced and transversely cut at 1 cm intervals; tissue blocks were obtained and 3μm thick sections stained with H&E and were studied on a general-purpose colour image processor in which a television camera was attached to a high resolution monitor, images on which could be processed and calculated through the microprocessor. They showed that in normal adult hearts myocardial fibre diameter were 9.9 ± 0.6 μm (mean ± sd) in the right ventricular free wall, 11.2 ± 0.6 to 12.3 ± 0.7 μm (mean ± sd) in the right ventricular side, middle and left ventricular side of the septum and 13.0 ± 0.7 to 11.2 ± 0.7 μm (mean ± sd) in the inner to outer side of the left ventricular free wall. Myocardial fibre diameter decreased from the inner to outer third of the left ventricular free wall and from the left ventricular to the right ventricular side of the septum with no significant difference between male and female patients. To minimise under-estimates of diameters, measurements were restricted to fibres containing nuclei and restricted to the short axis of fibres.

It was with the scope of ensuring that this variability was taken into consideration in conducting this study that the different areas of the ventricular walls were studied but no specific measurements from the inner to outer third of the ventricular walls were made. Indeed, the muscle fibres and nuclei were significantly bigger in the left ventricular walls as compared to the right ventricle in the controls but no significant variation was demonstrable among the different sites in the left ventricular walls in this series. The results for the diameters of muscle fibres in the controls in this study are fairly similar to those published by Hoshino et al (1983) but not with Fuster et al (1977) who used different techniques.

In these publications cited above, no attempts were made to estimate nuclear diameters or to assess interstitial tissue content.

Studies in both experimental animals and in humans have matched increased nuclear size within cardiac myocytes with the development and progression of cardiac hypertrophic changes (Linzbach, 1960; Astorri et al, 1977; Anversa et al, 1979; Olivetti et al, 1988; Gerdes et al, 1994). This appears to be a feature of the overall increase in size of the fibre and is not related to intrinsic alterations in the DNA content of the nucleus (Kellerman et al, 1992). Nuclear diameter and nuclear volume are therefore, useful parameters by which to assess changes in cardiac

myocyte size but these measurements appeared to have been largely disregarded by other workers (Grimm et al, 1970). The results for the measurement of the nucleus to muscle fibre ratio in this study also showed the enlargement of the cardiac muscle fibre nucleus occurred to the same extent as the muscle fibre.

Fujiwara et al (1983) attempted to clarify the pathogenesis of hypertrophied cardiomyopathy by an assessment of interstitial tissue space content. The method used was a video imager attached to a microprocessor and a printer, and the estimates of percentage area of transmural interstitial tissue were made semi-automatically. No attempts were made to exclude space occupied by non-connective tissue. No significant difference in the percentage area of the transmural interstitial space was identified in the heart studied, with this area ranging 28 to 55%, which is described as compatible with Fuster et al (1977). In this study interstitial tissue space (empty space) in the controls were 42.64% ± 3.93% (mean ± sd), excluding the connective tissue area which is 5.28% ± 1.58% (mean ± sd), is comparable with their findings. Fujiwara et al also found mean size of myocytes in their controls were 13 ± 1 μm to 14 ± 1 μm in the left ventricle. The mean size of myocyte in the left ventricle in this study controls were 12.32 ± 0.94 μm to 13.06 ± 1.12 μm which is comparable with their findings.

Tanaka et al (1986) studied distribution of myocardial fibrosis in hearts removed at autopsy from subjects with normal hearts and patients with hypertension and hypertrophied cardiomyopathy. 4 μm thick sections were stained with Masson's trichrome stain and directly enlarged by a photo enlarger to x10 magnification and all the areas stained blue were traced with a black pen and semi-automatically measured with a multipurpose colour image processor. The percentage area of fibrosis from 1.1 ± (0.5)% in normal hearts, rising to 2.6 (1.5)% in hypertensive hearts and 10.5 ± (4.3)% in hypertrophied cardiomyopathy. There was no correlation between the extent of fibrosis and septal thickness or with heart weight in any heart in hypertrophied cardiomyopathy and in normal hearts. This led to the conclusion that fibrosis was another feature of the pathology of hypertrophied cardiomyopathy but the pathogenesis of this fibrosis was not commented on in the study. By contrast, the percentage area of fibrosis in the normal hearts as measured in this study was 5.28 ± (1.58)%. Rose and Beck (1985) carried out a semiquantitative morphological assessment in their study of distribution of fibrosis in the hearts of 54 autopsy controls. The percentage are of fibrosis was 8.49 ± (7.11)%.

8.4.4 Correlation between Morphometry and Heart Weight

It would have been ideal if the findings in morphometry had matched the changes in heart weight particularly those of the separated right and in the left ventricles, with and without trimming of fat. Right ventricular increase in weight would have ideally matched the morphometric evidence of hypertrophy in myocytes and in myocytic nuclei. In this study the results for the diameters of muscle fibres and nuclei show some dependence on heart weight. However, given the various variables that may affect the weight of the heart and its chambers, this apparent lack of concordance between morphometry and heart weights is perhaps to be expected.

Enlargement of ventricular wall is a subtotal of increase in all its constituents mainly, muscle and connective tissue. It would therefore, be possible for a heart to enlarge at the same time that it shows a relative loss of myocytes and the proliferation of interstitial connective tissue. Myocyte cell loss occurs together with myocyte hypertrophy in the ageing heart of the rat (Anversa et al, 1986).

It appears therefore, that this situation may be pertinent to the alcoholic heart in which instance changes in thickness are a manifestation of both increased size of muscle fibres with no increase in numbers in association with increase connective tissue.

It is also a well known phenomenon that there is a considerable variability of muscle cell size within the myocardium of a patient, and even among immediately adjacent muscle cells in a tissue block, with normal sized cells may be found next to severely enlarged ones (Ferrans, 1982). This phenomenon is also found in experimental animals in cardiac hypertrophy (Smith and Bishop, 1985; Zimmer et al, 1990Liu et al, 1991 and 1991) and pronounced regional differences in myocyte size in the normal rat heart was demonstrated using very sophisticated measurements by Coulter Channelyzer system on isolated myocytes (Gerdes et al, 1986).

This is the reason why different areas of left ventricular myocardium were sampled from each of the heart in this series. No specific attempts were made to evaluate the degree of variation from the inner to the outer third of the myocardium.

8.5 Vascular Measurements

No attempts were made to correlate the actual number of vascular channels to the other morphological measurements already alluded to. It was thought that it would be impossible from the selected histological sections which were examined to obtain reasonably representative and meaningful ratios for this type of analysis.

Small vessel changes have been reported as part of the pathology of alcoholism of the heart. Burch and Giles (1977) studied smaller branches of the coronary arteries in alcoholic cardiomyopathy and found no major abnormalities apart from some luminal dilatation.

Factor (1976), however, in the study of patients under 45 years tabulated features of vascular oedema, sclerosis, inflammation and subendothelial thickening in 9 patients.

Burns and Hulewicz (1990) described changes in the small intra-myocardial vessels, particularly 'subendothelial humps' in a 72 year old chronic alcoholic man who died suddenly. His heart weighed 325 gm with no coronary arterial atheroma of significance.

The cases in this series were therefore looked at from the point of view of the vascular wall thickening. On the basis of measurements and calculations made of different variables of the arterial vascular channels e.g. area and diameters of lumen, intima and media etc., in the hearts of alcoholics, 65 tests of significance were carried out overall but there are only 5 significant results at the 5% level, and only one at the 1% level (see chapter 7, section 7.4 to 7.8) that would have been expected by chance alone. This suggests that there are no consistent differences in thickening of the vascular walls of small intra-myocardial arterial vessels.

The only suggestion that can be made from the statistical analysis of the vessel wall measurement is that intra-myocardial arterioles show some tendency to dilatation but little else of significance may be deduced from the other measurements.

The medial thickening identified in two areas are not matched by similarly statistically significant results in other parts of the myocardium. Interpretation of this data is therefore not possible.

8.6 Limitations of Techniques in the Study

The present study was based on autopsy material fixed in 10% buffered formal solution for variable periods and all data were collected from formalin fixed hearts. Information about physical activity, body size, age-dependent and sex-dependent changes in cardiac morphologic features may be important in the interpretation of normal hearts and this should be taken into account in the evaluation of the cardiac changes. No attempt was made to evaluate the possible mechanism by which alcohol exerts its toxic cellular effects. The use of myocardial biopsy in living patients is probably the only way by which direct information on acute toxic effect is obtainable.

The cross-sectional design of this study is another potential limitation, because the exact duration, type and quantity of alcohol abused were not known. It was however, a random sample from the population, selected only on the basis of presentation as a sudden unexpected death, but it should be noted that alcoholism was an accepted condition in these particular decedents.

For the measurement of the variables the semi-automatic computer-assisted morphometric method that was used is not totally free of observer and sampling error, although it is much easier, quicker, accurate and large numbers of measurements could be carried out within a short period of time compared to the graticule method.

8.7 Future study and Potential Clinical Applications

This case-control cross-sectional study is limited by the deficiency in the pre-death medical history, in that there has been assumption that apart from the pathologies caused by alcohol no other diseases which could affect the heart had

been present. The only way in which this hurdle could be overcome would be for an animal model in which alcohol is known to have an effect on the heart be used in the similar fashion. In such cases the exact volume of alcohol consumed and duration of their consumption could be accurately quantified.

Animals known to be absolutely healthy could be exposed on the controlled nutritional condition to a chronic level of ethanol and serial examination of cardiac musculature could be carried out to assess the progressive development of muscular, connective tissues and vascular changes. Similar morphometric equipment can be adopted for such study and in which the pathogenetic mechanisms could be investigated simultaneously, to identify histochemical, biochemical and ultrastructural changes and this would be done in a sequential fashion enabling a comparison to be made with the morphological findings.

In the human situation there may be scope for obtaining more frequently autopsies in cohorts of patients known to be chronic alcoholic but in whom accurate and comprehensive histories of alcohol abuse are available. Such patients would have attended clinics, and in whom the number of units of alcohol consumed per week, the actual beverages containing alcohol which were consumed, e.g. spirits, wines and beers, the exact number of years of the duration of alcohol abuse and the systemic biochemical abnormalities associated could be matched to the autopsy findings. In these patients specific investigations of cardiac size, rhythm, etc. would also have been made clinically on a regular and recurring basis and these data could also be correlated with the eventual findings of the heart at autopsy histology.

The morphological changes which this study has shown in the hearts of chronic alcohol abusers consolidate "previous observations" (Vikhert et al, 1986; Tsiplenkova et al, 1986; Stott et al, 1991; Preedy et al, 1993; Preedy and Richardson, 1994) that before cardiac symptoms and signs are identifiable clinically, the heart is already demonstrating definitive and quantifiable structural abnormalities. This information should further assist clinicians in their monitoring of patients with alcohol problem from the cardiological point of view. The contribution of alcohol to chronic heart complaints is further highlighted.

Chapter 9

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Appendix

A. Program I for Muscle and Connective tissue measurements

```
□Ó# INITIALISING
□
□loadlut "grey"
□clearall 0
□resetpar
□InitObj AREA,DMAX,DMIN
□InitField AREAP,REFAREA,TOTALAREA
□InitPDios POINTS
□InitPDios DISTANCE2P
□scalgeom 1,"leitzx25b/w",_OFF,_OFF
□
□# BACKGROUND CORRECTION
□
□tvon
□for i=1,i<7,i=i+1:write ""
□write "select the x25 lens"
□for i=1,i<7,i=i+1:write ""
□write "go to an empty area of the slide"
□pause
□tvinp 1
□shaddef 1,20
□
□# MAIN LOOP
□
□append=_OFF
□while 1
□clearallov
□loadlut "grey"
□tvon
□for i=1,i<7,i=i+1:write ""
□write "go to an area you want to measure"
□pause
□while 6
```

```
tvinp 1
shadcorr 1,2,20,_ON,0
loadlut "grey"
normim 2,2,1
enhcont 2,3,3,2
median 3,4,3
dis2lev 4,5,8,199,_ON,_ON,1
scrap 5,7,_OFF,0,200,_ON,_ON
fill 7,7
add 4,7,7,3
cutlink 7,8,5,0,_ON
dis2lev 8,8,135,255,_ON,_OFF,1
identify 8,9,_ON,_OFF
rejectobj 9,2,0
 ans="n"
for i=1,i<7,i=i+1: write ""
read "are you happy with this image [n]/y ",ans
if ans=="y":break
endwhile

# MUSCLE FIBRE MEASUREMENT

while 2
ans="n"
read "do you wnt to measure muscle fibre area [y]/n ",ans
if ans=="n":break
for i=1,i<7,i=i+1: write ""
 write "measuring muscle fibre area"
measfield 9,"muscle2",append
outlist "muscle2",_OFF
endwhile
for i=1,i<7,i=i+1: write ""

 while 3
loadlut "grey"
   read "do you want to measure muscle fibre diameter [y]/n ",ans
   if ans=="n":break
```

```
□   meas2p 1,"muscle3",append,_ON,-1
□outlist "muscle3",_OFF,_OFF
□ endwhile
□
□# CONNECTIVE TISSUE MEASUREMENT
□
□while 4
□ans="n"
□read "do you want to measure connective tissue area [y]/n ",ans
□if ans=="n":break
□loadlut "grey"
□  write "measuring area of interstitial connective tissue"
□tvon
□tvinp 1
□shadcorr 1,2,20,_ON,0
□normim 2,2,1
□dis2lev 2,3,0,137,_ON,_ON,1
□scrap 3,4,_OFF,0,10,_ON,_ON
□identify 4,4,_ON,_OFF
□measfield 4,"connect",append
□outlist "connect",_OFF
□endwhile
□append=_ON
□ans="y"
□read "do you want to print the histograms [y]/n ",ans
□if ans=="y" : break
□endwhile
□outhist
"muscle3","DISTANCE2P",15,_OFF,_OFF,0,_ON,_ON,0.00,100.00,100.00,"","
HISTOGRAM"
□outhist
"muscle2","AREAP",15,_OFF,_OFF,0,_ON,_ON,0.00,100.00,100.00,"","HIST
OGRAM"
□outhist
"connect","AREAP",15,_OFF,_OFF,0,_ON,_ON,0.00,100.00,100.00,"","HISTO
GRAM"
```

B. Program II for Nuclear studies

```
□Ó# INITIALISING
□
loadlut "grey"
□resetpar
□InitObj AREA,DMAX,DMIN
□InitField AREAP,REFAREA,TOTALAREA
□InitPDios POINTS
□InitPDios DISTANCE2P
□scalgeom 1,"leitzx25b/w",_OFF,_OFF
□
□# BACKGROUND CORRECTION
□
□tvon
□for i=1,i<7,i=i+1:write ""
□write "select the x25 lens"
□for i=1,i<7,i=i+1:write ""
□write "go to an empty area of the slide"
□pause
□tvinp 1
□shaddef 1,20
□
□# MAIN LOOP
□
□append=_OFF
□while 1
□loadlut "grey"
□tvon
□for i=1,i<7,i=i+1:write ""
□write "go to an area you want to measure"
□pause
□tvon
□tvinp 1
□shadcorr 1,2,20,_ON,0
□normim 2,2,1
```

□dis2lev 2,3,8,199,_ON,_ON,1

□scrap 3,4,_OFF,0,100,_ON,_ON

□add 2,4,5,3

□eraseinside 5,6,0

□dis2lev 6,7,150,255,_ON,_ON,1

□scrap 7,7,_OFF,0,100,_ON,_ON

□cutlink 7,7,3,255,_ON

□fill 7,7

□identify 7,8,_ON,_OFF

□rejectobj 8,2,0

□measobj 8,"nuclei",append

□outlist "nuclei",_OFF

□append=_ON

□endwhile

□outhist
"nuclei","AREA",15,_OFF,_OFF,0,_ON,_OFF,0.00,100.00,100.00,"","HISTOG
RAM"

□outhist
"nuclei","DMIN",15,_OFF,_OFF,0,_ON,_ON,0.00,100.00,100.00,"","HISTOG
RAM"

C. Program III for Blood Vessel studies

```
loadlut "grey"
□clearall 0
□resetpar
□resetlim
□resetvec "Extr2"
□measstop
□InitObj AREA,CPERIM,DMAX,DMIN,PERIM
□InitPDios POINTS
□InitPDios DISTANCE2P
□InitDios
AREA,CPERIM,DCIRCLE,DMAX,DMIN,FCIRCLE,LENGTH,PERIM
□scalgeom 1,"leitzx25b/w",_OFF,_OFF
□
□# SETTING UP DATABASES#
□
□#set up empty database for area#
□global LUMEN,IEL,EEL,INTIMA,MEDIA
□LUMEN=IEL=EEL=INTIMA=MEDIA=0.0
□db = "area"
□fv[] = LUMEN,IEL,EEL,INTIMA,MEDIA
□DBerase db
□DBcreate db,"fv"
□
□#set up empty database for DMAX#
□global DMAXLUM,DMAXIEL,DMAXEEL,DMAXMEDIA,DMAXINTIMA
□DMAXLUM=DMAXIEL=DMAXEEL=DMAXMEDIA=DMAXINTIMA=0.0
□dbase = "dmax"
□fv1[] = DMAXLUM,DMAXIEL,DMAXEEL,DMAXMEDIA,DMAXINTIMA
□DBerase dbase
□DBcreate dbase,"fv1"
□
□#set up empty database for DMIN#
□global DMINLUM,DMINIEL,DMINEEL,DMINMEDIA,DMININTIMA
□DMINLUM=DMINIEL=DMINEEL=DMINMEDIA=DMININTIMA=0.0
□dbase2 = "dmin"
```

```
□fv2[] = DMINLUM,DMINIEL,DMINEEL,DMINMEDIA,DMININTIMA
□DBerase dbase2
□DBcreate dbase2,"fv2"
□
□#set up empty database for perim#
□global LUMLEN,IELLEN,EELLEN
□LUMLEN=IELLEN=EELLEN=0.0
□dbase3 = "length"
□fv3[] = LUMLEN,IELLEN,EELLEN
□DBerase dbase3
□DBcreate dbase3,"fv3"
□
□#BACKGROUND CORRECTION#
□
□write "go to an empty area of the slide"
□tvon
□pause
□tvinp
□shaddef 1,11
□append=_OFF
□
□#MAIN LOOP#
□
□while 1
□while 2
□clovl 1
□measstop
□loadlut "grey"
□for i=1,i<7,i=i+1:write ""
□write "go to an area you want to measure"
□tvon
□pause
□tvinp 1
□shadcorr 1,1,11,_ON,0
□normim 1,1,1
□dis2lev 1,2,64,191,_ON,_ON,1
□scrap 2,3,_OFF,0,200,_ON,_ON
```

```
□write "draw around the outside of the blood vessel"
□eraseoutside 3,4,0
□scrap 4,4,_OFF,0,50,_ON,_ON,1
□write "click on the white of the vessel and then join any gaps"
□median 4,5,5
□cutlink 5,5,5,255,_ON
□fill 5,6
□xorim 5,6,7
□scrap 7,7,_OFF,0,200,_ON,_ON
□add 1,7,8,3
□write "draw around any inclusions in the blood vessel"
□eraseinside 8,8,0
□dis2lev 8,8,200,255,_ON,_OFF,1
□fill 8,8
□scrap 8,8,_OFF,0,200,_ON,_ON
□median 8,9,5
□identify 9,9,_ON,_OFF
□rejectobj 9,2,0
□ans = "y"
□read "are you happy with these images [y]/n",ans
□if (ans == "y") : break
□endwhile
□
□#LUMEN#
□
□measobj 9,"lumen",append
□outlist "lumen",_OFF
□
□#INTERNL ELASTIC LAMINA#
□
□display 6
□identify 6,6,_ON,_OFF
□measobj 6,"iel",append
□outlist "iel",_OFF
□
□#EXTERNAL ELASTIC LAMINA#
□
```

```
□loadlut "grey"
□display 1
□write "draw around the external elastic lamina"
□measdios 1,1,"eel",append,-1
□outlist "eel",_OFF
□
□ans = "y"
□read "do you want to calculate measurements",ans
□if (ans == "y") : break
□append=_ON
□endwhile
□
□DBclose "lumen"
□DBclose "iel"
□DBclose "eel"
□DBclose db
□DBclose dbase
□DBclose dbase2
□DBclose dbase3
□
□
□fv4[] = AREA,DMAX,DMIN,PERIM
□fv5[] = AREA,DMAX,DMIN,PERIM
□fv6[] = AREA,DMAX,DMIN,PERIM
□
□#opening and reseting databases#
□
□DBopen "lumen","fv4"
□DBtop "lumen"
□DBopen "iel","fv5"
□DBtop "iel"
□DBopen "eel","fv6"
□DBtop "eel"
□
□n=0
□
□while (3)
```

```
□
□n=n+1
□DBread "lumen"
□LUMEN=AREA
□DMAXLUM=DMAX
□DMINLUM=DMIN
□LUMLEN=PERIM
□if (_STATUS) : break
□DBread "iel"
□IEL=AREA
□DMAXIEL=DMAX
□DMINIEL=DMIN
□IELLEN=PERIM
□DBread "eel"
□EEL=AREA
□DMAXEEL=DMAX
□DMINEEL=DMIN
□EELLEN=LENGTH
□
□#Making database calculations#
□INTIMA = IEL-LUMEN
□MEDIA = EEL-IEL
□DMAXINTIMA = (DMAXIEL-DMAXLUM)/2
□DMAXMEDIA = (DMAXEEL-DMAXIEL)/2
□DMININTIMA = (DMINIEL-DMINLUM)/2
□DMINMEDIA = (DMINEEL-DMINIEL)/2
□
□DBopen db,"fv"
□DBappend db
□DBclose db
□DBopen dbase,"fv1"
□DBappend dbase
□DBclose dbase
□DBopen dbase2,"fv2"
□DBappend dbase2
□DBclose dbase2
□DBopen dbase3,"fv3"
```

□DBappend dbase3

□DBclose dbase3

□write "calculating...";n

□endwhile

□

□outlist db,_OFF

□outlist dbase,_OFF

□outlist dbase2,_OFF

□outlist dbase3,_OFF

□

□outhist
db,"LUMEN",15,_OFF,_OFF,0,_ON,_ON,157.92,3873.13,2.00,"","HISTOGRA
M"

□outhist
db,"INTIMA",15,_OFF,_OFF,0,_ON,_ON,33.50,101.75,2.00,"","HISTOGRAM
"

□outhist
db,"MEDIA",15,_OFF,_OFF,0,_ON,_ON,33.50,101.75,2.00,"","HISTOGRAM"

□outhist
dbase3,"IELLEN",15,_OFF,_OFF,0,_ON,_ON,33.50,101.75,2.00,"","HISTOGR
AM"

□outhist
dbase,"DMAXLUM",15,_OFF,_OFF,0,_ON,_ON,33.50,101.75,2.00,"","HISTO
GRAM"

□outhist
dbase2,"DMINLUM",15,_OFF,_OFF,0,_ON,_ON,33.50,101.75,2.00,"","HISTO
GRAM"

□outhist
dbase,"DMAXIEL",15,_OFF,_OFF,0,_ON,_ON,33.50,101.75,2.00,"","HISTOG
RAM"

□outhist
dbase2,"DMINIEL",15,_OFF,_OFF,0,_ON,_ON,33.50,101.75,2.00,"","HISTOG
RAM"

□outhist
dbase,"DMAXEEL",15,_OFF,_OFF,0,_ON,_ON,33.50,101.75,2.00,"","HISTO
GRAM"

□outhist
dbase2,"DMINEEL",15,_OFF,_OFF,0,_ON,_ON,33.50,101.75,2.00,"","HISTO
GRAM"
□outhist
dbase,"DMAXINTIMA",15,_OFF,_OFF,0,_ON,_ON,33.50,101.75,2.00,"","HIS
TOGRAM"
□outhist
dbase2,"DMININTIMA",15,_OFF,_OFF,0,_ON,_ON,33.50,101.75,2.00,"","HIS
TOGRAM"
□outhist
dbase,"DMAXMEDIA",15,_OFF,_OFF,0,_ON,_ON,33.50,101.75,2.00,"","HIS
TOGRAM"
□outhist
dbase2,"DMINMEDIA",15,_OFF,_OFF,0,_ON,_ON,33.50,101.75,2.00,"","HIS
TOGRAM"
□
□DBclose "lumen"
□DBclose "iel"
□DBclose "eel"
□DBclose db
□DBclose dbase
□DBclose dbase2
□DBclose dbase3
□
□endwhile